Iran, M.
09/854638

09/854638

[redacted] ENTERED AT 11:46:16 ON 24 APR 2002
E DEXTRAN/CN
L1 1 S E3
E LYSINE/CN 5
L2 2 S E3

- Key terms

[redacted]TERED AT 11:46:32 ON 24 APR 2002
L1 1 SEA FILE=REGISTRY ABB=ON PLU=ON DEXTRAN/CN
L2 2 SEA FILE=REGISTRY ABB=ON PLU=ON LYSINE/CN
L3 1614 SEA FILE=CAPLUS ABB=ON PLU=ON (96(W)WELL OR (MATRIX OR MATRIC?)(1W)LASER(2W)(IONIZ? OR IONIZ?) OR MALDI)(S)PLATE

L4 46 SEA FILE=CAPLUS ABB=ON PLU=ON L3 AND (POLYMER## OR DEXTRAN OR L1)
L11 14 SEA FILE=CAPLUS ABB=ON PLU=ON L4 AND (L2 OR LYSINE OR LYS OR LINK? OR SPACER OR CONJUGAT?)

L11 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:301222 CAPLUS
TITLE: Discovery of New Fluorescent Materials from Fast Synthesis and Screening of **Conjugated Polymers**
AUTHOR(S): Lavastre, Olivier; Illitchev, Ilya; Jegou, Gwenaelle; Dixneuf, Pierre H.
CORPORATE SOURCE: Institut de Chimie, UMR 6509 Universite de Rennes 1 - CNRS, Rennes, 35042, Fr.
SOURCE: Journal of the American Chemical Society ACS ASAP
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A combinatorial approach was developed for the synthesis and the screening of a large variety of **conjugated polymers**. The parallel synthesis of 96 polyaryleneethynylene derivs. was performed on a 12 .times. 8 format from diethynyl and dibromoaryl building blocks, via a palladium-catalyzed carbon-carbon coupling reaction. The qual. distinction between fluorescent and nonfluorescent **polymers** as well as between the different emission colors were obtained from a simple visual test or via a **96-well plate** reader spectrofluorimeter. New solid-state blue-emitting **polymers** were detected.

L11 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:108215 CAPLUS
DOCUMENT NUMBER: 136:196454
TITLE: High-performance microtiter plates for immunosorbent assays made of renewable resources: polylactic acid biopolymer as a substitute for synthetic polystyrene
AUTHOR(S): Bouche, Fabienne B.; Schecklies, Elvira; Muller, Claude P.
CORPORATE SOURCE: Laboratoire National de Sante, Luxembourg, L-1011, Luxembourg
SOURCE: Clinical Chemistry (Washington, DC, United States) (2002), 48(2), 378-380
CODEN: CLCHAU; ISSN: 0009-9147

Searcher : Shears 308-4994

PUBLISHER: American Association for Clinical Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The injection-molding process commonly used for polystyrene (PS) was adapted to the polylactic acid (PLA) **polymer** to produce an exptl. batch of **96-well** microtiter **plates** from suitable PLA formulations. The performance of these plates in an ELISA was compared with conventional com. PS plates. These microtiter plates were used to analyze a panel of 426 human sera. Recombinant measles virus hemagglutinin protein (H-ELISA) was used as antigen for the detection of measles-specific IgG. Raw absorbance of the serum reactivity with the H protein and the net absorbance values were assessed. Despite a somewhat narrower dynamic range of the PLA plate, substituting the conventional com. PS-based ELISA plates by the PLA prototype plates did not compromise the specificity or the sensitivity of the assay. Both neg. and pos. samples had a >99% chance of being tested correctly with the PLA-based H-ELISA. The absorbances of both types of plates were considerably correlated whether net or raw values were considered for the PLA plates, which were also shown to bind various antigens, including antibodies and peptides.
REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:864155 CAPLUS
DOCUMENT NUMBER: 136:114152
TITLE: An immunoassay for terbutryn using direct hapten **linkage** to a glutaraldehyde network on the polystyrene surface of standard microtiter plates
AUTHOR(S): Holthues, Heike; Pfeifer-Fukumura, Ursula; Hartmann, Iris; Baumann, Wolfram
CORPORATE SOURCE: University of Mainz, Institute of Physical Chemistry, Mainz, 55128, Germany
SOURCE: Fresenius' Journal of Analytical Chemistry (2001), 371(7), 897-902
CODEN: FJACES; ISSN: 0937-0633
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Aminobutylamino-4-ethylamino-6-isopropylamino-1,3,5-triazine (ABA-atrazine) has been synthesized and used as a coating hapten in an immunoassay with a monoclonal antibody against terbutryn. Coating was achieved by covalently **linking** ABA-atrazine to a glutaraldehyde **polymer** network directly bound to the polystyrene surface of a std. **96-well** microtiter **plate**. The assay was carefully optimized. In particular, the coating hapten concn. had a strong effect on the ELISA sensitivity. By including a pre-incubation step a low test midpoint (IC50-value) of 0.130 .mu.g L-1 was achieved. As far as we are aware this is the most sensitive ELISA for terbutryn yet reported. The coating-hapten-format presented is proposed as generally applicable, because the glutaraldehyde-modified microtiter plate surface enables stable immobilization of a broad variety of coating haptens.
REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE

FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:662415 CAPLUS
DOCUMENT NUMBER: 135:344885
TITLE: New **polymers** and dyes the combinatorial way
AUTHOR(S): Bradley, Mark
CORPORATE SOURCE: Department of Chemistry, University of Southampton, Southampton, UK
SOURCE: Polymer Preprints (American Chemical Society, Division of Polymer Chemistry) (2001), 42(2), 629
CODEN: ACPPAY; ISSN: 0032-3934
PUBLISHER: American Chemical Society, Division of Polymer Chemistry
DOCUMENT TYPE: Journal; (computer optical disk)
LANGUAGE: English
AB Combinatorial methodologies were used for synthesis, screening, and anal. of dyes and **polymers**, ranging from beads produced by suspension **polymn.** to mol. imprinted **polymers**, and by combinatorial radiation grafting methods. **Polymers** were prepd. from styrene, divinylbenzene, chloromethylstyrene, and various poly(ethylene glycol) (PEG) via rapid parallel synthesis (6-12 reactions) using a specially designed reactor. The resins showed good solvation profiles in a broad range of solvents and good chem. and mech. stability. Mol. imprinted **polymers** (MIPS) were prepd. in a **96 well plate** format from acrylic monomers, porogens and cross-**linkers**; the materials were screened following **polymn.** by automated HPLC directly from the **plate**. Using the combinatorial methods, optimum MIPS were identified in a rapid manner and were subsequently prepd. in bulk for large scale evaluation by more conventional HPLC anal. Resin based diazonium species, which were remarkably stable (weeks at -20.degree.) were used to prep. a library of azo dyes in a few days using parallel reactions and combinatorial methodologies.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:353284 CAPLUS
DOCUMENT NUMBER: 135:192282
TITLE: Polymerized lipid vesicles as colorimetric biosensors for biotechnological applications
AUTHOR(S): Jelinek, R.; Kolusheva, S.
CORPORATE SOURCE: Department of Chemistry, Stadler Minerva Center for Mesoscopic Macromolecular Engineering, Ben Gurion University of the Negev, Beer Sheva, 84105, Israel
SOURCE: Biotechnology Advances (2001), 19(2), 109-118
CODEN: BIADDD; ISSN: 0734-9750
PUBLISHER: Elsevier Science Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with numerous refs. Supramol. chem. assemblies composed of

polydiacetylene (PDA) exhibit rapid colorimetric transitions upon specific interactions with a variety of biol. analytes in aq. solns. Among the analytes that give rise to the unique blue-red color changes are lipophilic enzymes, antibacterial peptides, ions, antibodies, and membrane penetration enhancers. The chem. assemblies include **conjugated** PDA, responsible for the chromatic transitions, and the mol. recognition elements, which are either chem. or phys. assocd. with the PDA. Thus, by incorporation of specific recognition elements, the system can be designed in ways allowing for highly selective identification of analytes. In particular, receptors and epitopes can be incorporated within the sensor assembly, which then det. the specificity of the colorimetric transitions. The PDA-based mol. assemblies are robust and can be readily applied to diagnosis of physiol. mols. and for rapid screening of chem. and biol. libraries, for example, in **96 well-plate** platforms.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:594243 CAPLUS
DOCUMENT NUMBER: 133:307246
TITLE: Generation of environmentally compatible **polymer** libraries via combinatorial biocatalysis
AUTHOR(S): Dordick, J. S.; Kim, D.; Wu, X.
CORPORATE SOURCE: Department of Chemical Engineerign, Rensselaer Polytechnic Institute, Troy, NY, 12180, USA
SOURCE: Polymer Preprints (American Chemical Society, Division of Polymer Chemistry) (2000), 41(2), 1847-1848
CODEN: ACPPAY; ISSN: 0032-3934
PUBLISHER: American Chemical Society, Division of Polymer Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A methodol. for rapid synthesis and anal. of novel **polymeric** entities is developed. The use of biocatalysis ensures that high selectivity and potentially unique properties are generated, which can lead to the development of novel structure-function relationships among the **polymers** generated. The **polymeric** libraries generated can be screened for different properties (Tg and Tm) in addn. to mol. wt. Utilizing the instrumentation and techniques (i.e., **96-well plates**, **plates** readers, and microgram anal.) already in place by the combinatorial chem. field, the rapid screening and identification of potentially useful **polymers** can easily be managed. Moreover, the library was synthesized by nature's own catalyst and is comprized of simple ester **linkages**; therefore the **polymers** are expected to be biodegradable and environmentally benign.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:220642 CAPLUS

DOCUMENT NUMBER: 133:55411
TITLE: Microspheres for medical diagnostics: Part 2: specific tests and assays
AUTHOR(S): Bangs, Leigh B.
CORPORATE SOURCE: Bangs Laboratories Inc, Fishers, IN, USA
SOURCE: Microspheres, Microcapsules & Liposomes (1999), 2(Medical & Biotechnology Applications), 71-96
CODEN: MMLIFU; ISSN: 1461-1732
PUBLISHER: Citus Books
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 51 refs. Latex agglutination tests were invented in 1956, and new test formats are still being devised. Latex particles or microspheres are now also used as supports for sandwich tests in, for example, particle capture enzyme **linked** immunosorbent tests and assays (ELISA). Dyed microspheres now function as color tags in over-the-counter pregnancy tests, as well as in lab. tests for everything from pregnancy to drugs of abuse. Technol. creativity has expanded diagnostic opportunities from qual. yes/no tests to quant. assays. Particle (microsphere) agglutination is monitored by spectrophotometers or nephelometers in tubes or **96-well plates**, and color intensities on solid surfaces like filters are measured by reflectometers. Both plain and magnetic **polymer** particles are used in immunoassay instruments with, among others, radiometric, fluorometric and chemiluminescence detectors. The state of art description of these tests and assays and related technologies is discussed in this chapter.

REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:187951 CAPLUS
DOCUMENT NUMBER: 133:37658
TITLE: Determination of MAG-Camptothecin, a new **polymer**-bound Camptothecin derivative, and free Camptothecin in dog plasma by HPLC with fluorimetric detection
AUTHOR(S): Fraier, D.; Frigerio, E.; Brianceschi, G.; Casati, M.; Benecchi, A.; James, C.
CORPORATE SOURCE: Drug Metabolism Research, Pharmacokinetics and Metabolism Department, Pharmacia and Upjohn, Nerviano, Milan, 20014, Italy
SOURCE: Journal of Pharmaceutical and Biomedical Analysis (2000), 22(3), 505-514
CODEN: JPBADA; ISSN: 0731-7085
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A high throughput, selective and sensitive high-performance liq. chromatog. (HPLC) method for the detn. of a water-sol. **polymer**-bound Camptothecin **conjugate** (MAG-CPT) and Camptothecin (CPT) in dog plasma has been developed and validated. The method involved the anal. of free and total CPT (free+ **polymer**-bound). Free CPT (intact lactone plus carboxylate) was extd. from acidified plasma using Oasis SPE material in **96-well plates**. For the assay of the

total CPT, plasma proteins were first pptd. with methanol in a **96-well plate** contg. a 10-.mu.m melt blown polypropylene membrane. The methanolic supernatant was sepd. and collected into a second **96-well plate** by simply applying vacuum to the **plate**. After hydrolysis at pH 9.8 for 18 h and re-acidification, samples were injected directly from the collection plate onto the HPLC system. MAG-CPT concn. was then calcd. by subtraction of free from total CPT. The LLOQs of the method were 1.17 ng/mL for free CPT and 103.10 ng/mL (as CPT equiv.) for MAG-CPT using 0.1 and 0.05 mL of plasma, resp. Linearity, precision, accuracy and recovery of the method were evaluated. The stability of MAG-CPT in plasma alone and after its stabilization was carefully evaluated. No interference from blank dog, mouse and human plasma was obsd. The suitability of the method for in vivo samples was assessed by the anal. of samples obtained from dogs that had received a single and 5-day repeated dose of MAG-CPT.

REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:117236 CAPLUS
DOCUMENT NUMBER: 132:148738
TITLE: Calibration device for an optical microtiter plate reading apparatus
INVENTOR(S): Beumer, Thomas Augustinus Maria; Carpay, Wilhelmus Marinus
PATENT ASSIGNEE(S): Akzo Nobel N.V., Neth.
SOURCE: PCT Int. Appl., 17 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000008440	A1	20000217	WO 1999-EP5579	19990802
W: AU, CA, ID, JP, KR, US, ZA				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9955096	A1	20000228	AU 1999-55096	19990802
PRIORITY APPLN. INFO.:			EP 1998-202667 A	19980807
			WO 1999-EP5579 W	19990802

AB The present invention relates to a calibration device for an optical microtiter plate reading app., such as used for Enzyme **Linked** Immuno Sorbent Assays. The calibration device comprises a plurality of optically transparent areas having solid outer surfaces. According to the invention at least one area comprises a section having a beam of light diverging property, such as a concavely curved surface. The calibration device allows for assessing the quality of a plate reader for performing certain (immuno)assays. A calibration device was manufd. by pouring a mixt. of polyester molding resin and hardener in wells of a std. polystyrene microtiter **plate** with **96 wells**. The curvature of the resulting meniscus in each well was detd. using a measuring microscope.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:620714 CAPLUS
DOCUMENT NUMBER: 131:334216
TITLE: Direct **matrix**-assisted **laser** desorption/**ionization** mass spectrometric analysis of glycosphingolipids on thin layer chromatographic **plates** and transfer membranes
AUTHOR(S): Guittard, Joelle; Hronowski, Xiaoping L.; Costello, Catherine E.
CORPORATE SOURCE: Mass Spectrometry Resource, Departments of Biochemistry and Biophysics, Boston University School of Medicine, Boston, MA, 02118-2526, USA
SOURCE: Rapid Commun. Mass Spectrom. (1999), 13(18), 1838-1849
CODEN: RCMSEF; ISSN: 0951-4198
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Results are reported for anal. by **matrix**-assisted **laser** desorption/**ionization** time-of-flight mass spectrometry (**MALDI**-TOFMS) of native glycosphingolipids (GSLs) after development on thin layer chromatog. **plates** and after heat transfer of the GSLs from the **plates** to several types of **polymer** membranes. The spectral quality is better for membrane-bound analytes, in terms of sensitivity, mass resoln. and background interference. The sensitivity gain compared with liq. secondary ion mass spectrometry (LSIMS) of GSLs on thin layer plates is 1-2 orders of magnitude (detection limits of 5-50 pmol vs. 1-10 nmol). Resoln. and mass accuracy (0.1%) are limited by the irregular membrane surfaces and this effect cannot be entirely compensated by delayed extn. The best results were obtained with a polyvinylidene difluoride (PVDF) P membrane, with irradn. from a nitrogen laser. Although the Nafion membrane could not be used for mol. wt. profiling, its acidic character led to sample hydrolysis at the glycosidic **linkages**, thus yielding a series of fragments that could be used to det. the sequence of carbohydrate residues. Structural information could also be obtained by post-source decay (PSD) expts. on mass-selected precursor ions. Samples contg. both neutral and acidic components were characterized in a 1:1 combination of 2,5-dihydroxybenzoic acid and 2-amino-5-nitropyridine. GSLs that exhibited binding to antibodies in an overlay assay on the TLC **plate** were transferred to membranes and analyzed by **MALDI**-TOFMS without interference from the antibody or the salts and buffers used during the binding and visualization steps. Taking advantage of the insights into sample prepn. gained from these studies, future research will extend this approach to anal. by matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry (MALDI-FTICRMS) with an external ion source.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:329377 CAPLUS
DOCUMENT NUMBER: 131:138857
TITLE: Measuring DNA adducts by immunoassay (ELISA)
AUTHOR(S): Tilby, Michael J.
CORPORATE SOURCE: Cancer Research Unit, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, UK
SOURCE: Methods in Molecular Medicine (1999), 28(Cytotoxic Drug Resistance Mechanisms), 121-128
CODEN: MMMEFN
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An assay is described that measures DNA adducts of carcinogens and anticancer drugs using an immunoassay. The present protocol concerns antibodies raised against drug-modified **polymeric** DNA and although it attempts to be of general use, it is illustrated by including specific details relevant to the use of a rat monoclonal antibody that recognizes cisplatin-induced DNA modifications. This is a competitive ELISA (ELISA) involving competition between a const. amt. of antigen bound to the wells of a **96-well plate** and a variable quantity and/or quality of antigen in soln. These compete for an invariant small quantity of antibody in soln. The amt. of antibody that binds to the immobilized antigen is measured and expressed as a percentage of the amt. that binds in the absence of competing dissolved antigen. The sensitivity of detection of the competing antigen generally appears to be less than that of the immobilized antigen. Using this assay system, the authors have achieved sensitivities (50% inhibition) of 30 and 2 fmol/assay well for melphalan and cisplatin adducts on DNA resp. The properties of the antibodies being used are clearly a major factor in detg. the sensitivity that can be achieved in an assay.
REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:228098 CAPLUS
DOCUMENT NUMBER: 130:269221
TITLE: Method for separation of complex substance mixtures by solid phase extraction for use in the search for biologically active substances and for analytical purposes
INVENTOR(S): Grabley, Susanne; Schmid, Ingrid; Sattler, Isabel; Thiericke, Ralf
PATENT ASSIGNEE(S): Hans-Knoell-Institut fuer Naturstoff-Forschung e.V., Germany
SOURCE: Ger. Offen., 12 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

DE 19743176 A1 19990401 DE 1997-19743176 19970930
WO 9916526 A1 19990408 WO 1998-DE2907 19980930
W: CA, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
PRIORITY APPLN. INFO.: DE 1997-19743176 19970930

AB A solid phase extn. process is described having multiple elution stages for fractionation and sepn. of complex mixts., e.g., supernatants of microbial cultures, plant derived substances, animal cell products or metabolites, combinatorial libraries, biol. fluids, body fluids, or clin. samples. In each sepn. stage, adsorbent resins, reversed phase or liq. chromatog. stationary phases, or special adsorbents or chromatog. media are used. In an example, supernatants from Streptomyces cultures were fractionated using a polystyrene copolymer adsorbent resin with a 7-stage elution method using mixts. of MeOH and ammonium acetate with various MeOH concns. as eluants; then the recovered fractions were fractionated using a C8 endcapped reversed phase chromatog. stationary phase with a multistage elution process. The resulting fractions were received directly into a **96-well** microtiter **plate** for screening for activity toward progesterone receptors.

IT **56-87-1DP**, L-**Lysine**, combinatorial reaction products
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(complex mixt. sepn. by solid phase extn. with multistage elution process)

L11 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:627339 CAPLUS
DOCUMENT NUMBER: 113:227339
TITLE: A procedure for productive coupling of synthetic oligonucleotides to polystyrene microtiter wells for hybridization capture
AUTHOR(S): Running, J. A.; Urdea, M. S.
CORPORATE SOURCE: Chiron Corp., Emeryville, CA, 94608, USA
SOURCE: BioTechniques (1990), 8(3), 276, 279
CODEN: BTNQDO; ISSN: 0736-6205
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A procedure was developed for coating **96-well** microtiter **plates** for nucleic acid hybridization assay. Such soln. phase probe hybridization method involves synthetic oligonucleotides with single-strand extensions that can be used to capture the probe-target complex to a solid support and to label the target through a controlled networking of addnl. synthetic probes. Nearly all of the solid phase probe was available for hybridization.

L11 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1985:198724 CAPLUS
DOCUMENT NUMBER: 102:198724
TITLE: Bioassay of parathyroid hormone
INVENTOR(S): Lindall, Arnold W.; Elting, James J.
PATENT ASSIGNEE(S): Immuno Nuclear Corp., USA
SOURCE: U.S., 13 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 4508828	A	19850402	US 1983-477253	19830321

AB A method is given for the extn., concn., and bioassay of bioactive parathyroid peptides in biol. or other fluids. Parathyroid hormone (PTH) [9002-64-6] peptides are extd. and concd. from biol fluids by a H2O-insol. support-antibody matrix specific to the N-terminal region. The bioassay is conducted with a cell or tissue ext. contg. adenylate cyclase-coupled PTH receptors and the generated cAMP is measured by a described double antibody RIA. Thus, antibodies are raised to human PTH1-34 in chicken, and after (NH4)2SO4 purifn. are **conjugated** to activated agarose or activated cross-**linked dextran.** Bioactive PTH peptides are extd. from biol fluids, e.g. serum or plasma by affinity chromatog. with the support-antibody matrix specific to the N- terminal region. After adsorption to antibody matrix, the PTH peptides are eluted with 0.025 N HCl and the eluates are lyophilized and frozen. The dried eluates are made up to vol with the bioassay medium and the bioassay is performed with a rat osteogenic sarcoma cell culture in a std. **96-well** tissue culture **plate.** CAMP generated by std. levels of bovine PTH1-84 were compared with that of the exptl. samples in detg. PTH concn. The major improvement of the method is the concn. of the sample by affinity chromatog.

[redacted]LINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, [redacted]APIO' ENTERED AT 11:54:47 ON 24 APR 2002)

L[redacted] 31 S L11

[redacted] DUPLICATES REMOVED)

L13 ANSWER 1 OF 22 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-171026 [22] WPIDS
CROSS REFERENCE: 2000-423420 [36]; 2001-300278 [30]
DOC. NO. CPI: C2002-052770
TITLE: Promoting growth of bone, ligament or cartilage in a mammal, involves administering to the mammal a protein which comprises growth factor domain of zvegf3 protein, a homolog of platelet-derived growth factor.
DERWENT CLASS: B04 D16
INVENTOR(S): GILBERTSON, D G; HART, C E
PATENT ASSIGNEE(S): (GILB-I) GILBERTSON D G; (HART-I) HART C E
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2002004225	A1	20020110	(200222)*		31

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2002004225	A1 Provisional	US 1998-111173P	19981207

Provisional	US 1999-142576P	19990706
Provisional	US 1999-161653P	19991021
Provisional	US 1999-165255P	19991112
CIP of	US 1999-457066	19991207
Provisional	US 2000-193723P	20000331
	US 2001-823033	20010329

PRIORITY APPLN. INFO: US 2001-823033 20010329; US 1998-111173P 19981207; US 1999-142576P 19990706; US 1999-161653P 19991021; US 1999-165255P 19991112; US 1999-457066 19991207; US 2000-193723P 20000331

AN 2002-171026 [22] WPIDS

CR 2000-423420 [36]; 2001-300278 [30]

AB US2002004225 A UPAB: 20020409

NOVELTY - Promoting (M) growth of bone, ligament or cartilage and stimulating proliferation of osteoblasts or chondrocytes in a mammal, comprises administering to the mammal a composition containing a dimeric protein (DP) which comprises growth factor domain comprising residues 235-345 of human or mouse zvegf3 protein of 345 (S1) amino acids fully defined in the specification, and a delivery vehicle.

DETAILED DESCRIPTION - Promoting growth of bone, ligament or cartilage in a mammal, involves administering to the mammal a protein which comprises growth factor domain of zvegf3 protein, which is a homolog of platelet-derived growth factor. Alternatively, in in vitro method, cartilage growth is promoted by culturing chondrocytes ex vivo in the presence of DP under conditions, where the chondrocytes proliferate, and placing the cultured chondrocytes into a mammal where cartilage is to be grown. Proliferation or differentiation of osteoblasts, osteoclasts, chondrocytes or bone marrow stem cells is promoted by culturing the cells in an effective amount of DP.

ACTIVITY - Osteopathic.

MECHANISM OF ACTION - Promoter of cell proliferation and differentiation. Recombinant zvegf3 was analyzed for mitogenic activity on human aortic smooth muscle cells (HAoSMC) and human umbilical vein endothelial cells (HUVEC). HAoSMC and HUVEC were plated at a density of 5000 cells/well in **96-well** culture **plates** and grown for 24 hours in DMEM (Dulbecco's Modified Eagle medium) containing 10% fetal calf serum at 37 deg. C. Cells were quiesced by incubating them for 24 hours in serum-free DMEM/Ham's F-12 medium containing insulin (5 micro g/ml), transferrin (20 micro g/ml) and selenium (16 pg/ml) (ITS). Test samples consisted of either conditioned media (CM) from adenovirally-infected HaCaT human keratinocyte cells expressing full-length zvegf3, purified growth factor domain expressed in BHK cells, or control media from cells infected with parental adenovirus. The control CM was generated from HaCaT cells infected with a parental green fluorescent protein-expressing adenovirus and treated identically to the zvegf3 CM. Purified protein in a buffer containing 0.1% bovine serum albumin (BSA) was serially diluted into ITS medium at concentrations of 1 micro g/ml-1 ng/ml and added to the test **plate**. A control buffer of 0.1% BSA was diluted identically to the highest concentration of zvegf3 protein and added to the **plate**. For measurement of (3H) thymidine incorporation, 20 micro l of a 50 micro Ci/ml stock in DMEM was

added directly to the cells, for a final activity of 1 micro Ci/well. Mitogenic activity was assessed by measuring the uptake of (3H) thymidine. The results demonstrated that zvegf3 CM had approx. 1.5-fold higher mitogenic activity on HAoSM cells over control CM, and purified protein caused a maximal 1.8-fold increase in (3H) thymidine incorporation over the buffer control. zvegf3 CM had no mitogenic activity on HUVEC compared to the control CM, and purified protein caused a maximal 1.3-fold increase in (3H) thymidine incorporation over the buffer control.

USE - (M) is useful for promoting growth of bone, ligament or cartilage in a mammal, where the composition is administered at a site of a bony defect, preferably a fracture, bone graft site, implant site, or periodontal pocket, and for stimulating proliferation of osteoblasts or chondrocytes in a mammal. (M) is further useful for promoting proliferation of osteoblasts, osteoclasts, chondrocytes or bone marrow stem cells, where the bone marrow stem cells are harvested from a patient prior to culture (claimed). The method is therefore useful for treating osteoporosis. Dwg.0/2

L13 ANSWER 2 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:207328 BIOSIS
DOCUMENT NUMBER: PREV200200207328
TITLE: WaaP of Pseudomonas aeruginosa is a novel eukaryotic type protein-tyrosine kinase as well as a sugar kinase essential for the biosynthesis of core lipopolysaccharide.
AUTHOR(S): Zhao, Xin; Lam, Joseph S. (1)
CORPORATE SOURCE: (1) Canadian Bacterial Diseases Network, Department of Microbiology, University of Guelph, Guelph, Ontario, N1G 2W1: jlam@uoguelph.ca Canada
SOURCE: Journal of Biological Chemistry, (February 15, 2002) Vol. 277, No. 7, pp. 4722-4730. http://www.jbc.org/. print.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English

AB WaaP of P. aeruginosa is a crucial sugar kinase that phosphorylates HepI in the inner core region of lipopolysaccharide (LPS). WaaP shares homology with eukaryotic protein kinases in the conserved functional motifs (I-IX), indicating that it is also a protein kinase. This interpretation is substantiated by several lines of evidence including the following: (i) site-directed mutagenesis on catalytic domain residues abrogated the protein kinase activity; (ii) positive reaction in immunoblotting with anti-phosphotyrosine monoclonal antibody PY20; (iii) matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and proteolytic peptide mapping showing excess mass equivalent to eight phosphate substituents on the tyrosine residues in WaaP; and (iv) WaaP is capable of catalyzing tyrosine self-phosphorylation as well as phosphorylating an exogenous synthetic co-**polymer** poly(Glu, Tyr). Thus, WaaP possesses dual kinase functions, and it utilizes a catalytic mechanism similar to that of the eukaryotic protein kinases. WaaP was localized to the cytoplasm, suggesting that phosphorylation of the LPS core occurred prior to translocation to the periplasm and attachment of O-antigen. A chemiluminescence-based enzyme-**linked** immunosorbent assay (ELISA) was developed to measure the kinetics of the WaaP sugar kinase activity,

and the results showed that the Km was 0.22 mM for ATP and 14.4 muM for hydrofluoric acid-treated LPS, Vmax was 408.24 pmol min-1, and kcat was 27.23 min-1.

L13 ANSWER 3 OF 22 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-188286 [24] WPIDS
DOC. NO. CPI: C2002-058100
TITLE: New prodrug compound useful for the treatment of e.g. cancer comprises a therapeutic agent, an oligopeptide, a stabilizing group and optionally a **linker**.
DERWENT CLASS: A96 B04 B05
INVENTOR(S): GANGWAR, S; LOBL, T J; NIEDER, M H; PICKFORD, L B; YARRANTON, G T
PATENT ASSIGNEE(S): (CORI-N) CORIXA CORP
COUNTRY COUNT: 96
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001095943	A2	20011220	(200224)*	EN	89

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001095943	A2	WO 2001-US18857	20010611

PRIORITY APPLN. INFO: US 2000-211686P 20000614
AN 2002-188286 [24] WPIDS
AB WO 200195943 A UPAB: 20020416
NOVELTY - A prodrug compound comprises a therapeutic agent capable of entering a target cell, an oligopeptide, a stabilizing group and (optionally) a **linker** not cleavable by thimet oligopeptidase (TOP).
DETAILED DESCRIPTION - A prodrug compound (A) comprises:
(1) a therapeutic agent capable of entering a target cell;
(2) an oligopeptide of formula (AA)n-AA3-AA2-AA1 (I);
(3) a stabilizing group; and
(4) optionally, a **linker** group not cleavable by TOP.
AA = an amino acid;
n = 0 or 1;
AA3 = isoleucine; and
AA1, AA2 = an amino acid;
provided that when n is 1 then (AA)n is an amino acid AA4.
The oligopeptide is directly **linked** to the stabilizing group at a first attachment site of the oligopeptide and is directly **linked** to the therapeutic agent or indirectly **linked** through the **linker** group to the therapeutic agent at a second attachment site of the oligopeptide. The

stabilizing group hinders cleavage of the compound by enzymes present in whole blood. The compound is cleavable by an enzyme associated with the target cell such as TOP.

INDEPENDENT CLAIMS are also included for the following:

(1) designing a prodrug for administration to a patient involving:

(a) identifying (I);

(b) **linking** (I) at the first attachment site of the oligopeptide to the stabilizing group; and

(c) directly or indirectly **linking** (I) to the therapeutic agent at the second attachment site of (I). Steps (b) and (c) may be performed in any order or concurrently and further where a **conjugate** is formed by performance of steps (a)-(c);

(d) testing if the **conjugate** is cleavable by TOP;

(e) testing if the **conjugate** is stable in whole blood; and

(f) selecting the **conjugate** as a prodrug if the **conjugate** is resistant to cleavage by TOP and stable in whole blood;

(2) an article of manufacture for diagnosis or assay comprising:

(a) a compound containing a marker, (I), the stabilizing group and optionally, a **linker** group not cleavage by TOP, and

(b) optionally at least one reagent useful in the detection of the marker; and

(3) a method of removing free therapeutic agent comprising:

(a) coupling an optionally protected stabilizing group-oligopeptide **conjugate** with the free therapeutic agent;

(b) contacting the reactants of step (a) with a **polymeric** resin to bind free therapeutic agent remaining after step (a) and to form a therapeutic agent-**polymeric** resin complex, and

(c) removing the therapeutic agent, **polymeric** resin complex.

ACTIVITY - Cytostatic; Antiinflammatory.

Adherant cells, LNCaP (prostate carcinoma) were cultured in DMEM media containing 10% heat inactivated fetal calf serum (FCS). On the day of the study the cells were detached from the **plate** with a trypsin solution. The collected cells were washed and re-suspended at a concentration of 0.25 multiply 106 cells/ml in DMEM containing 10% FCS. 100 micro l of cell suspension were added to **96 well plates** and the **plates** were incubated for 3 hours to allow the cells to adhere. Following the incubation, serial dilutions of doxorubicin (comparative) or Suc- beta Ala-Ile-Ala-Leu-Dox (test compound) were made and 100 micro l of the test/comparative compounds were added per well. The **plates** were then incubated for 24 hours, pulsed with 10 micro l of a 100 micro Ci/ml 3H-thymidine and incubated for an additional 24 hours. The **plates** were harvested and counted. IC50 values for the test and comparative compound when tested in LNCaP were 0.19 and 0.016 micro M respectively.

MECHANISM OF ACTION - Gene therapy.

USE - For decreasing toxicity of the therapeutic agent; for the treatment of a medical condition such as cancer, neoplastic disease, tumor, inflammatory or infectious disease in a patient (all

claimed); and for the treatment of breast, colorectal, liver, lung, prostrate, ovarian, brain and pancreatic cancers.

ADVANTAGE - The compounds display a high specificity of action, a reduced toxicity, an improved stability in blood especially relative to prodrugs of similar structure.

Dwg.0/17

L13 ANSWER 4 OF 22 WPIDS (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: 2001-355268 [37] WPIDS
DOC. NO. CPI: C2001-110059
TITLE: Screening for a functional protein variant with reduced antibody binding, by analyzing cell samples transformed by a diversified library of variants of relevant protein backbone for antibody binding and functionality of the variants.
DERWENT CLASS: A96 B04 D16
INVENTOR(S): ERNST, S; PEDERSEN, H; ROGGEN, E L
PATENT ASSIGNEE(S): (NOVO) NOVOZYMES AS; (ERNS-I) ERNST S; (PEDE-I) PEDERSEN H; (ROGG-I) ROGGEN E L
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001031989	A2	20010510	(200137)*	EN	72
AU 2001018522	A	20010514	(200149)		
US 2002019009	A1	20020214	(200214)		

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001031989	A2	WO 2000-DK682	20001208
AU 2001018522	A	AU 2001-18522	20001208
US 2002019009	A1 Provisional	US 1999-170743P	19991214
		US 2000-733485	20001208

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001018522	A Based on	WO 200131989

PRIORITY APPLN. INFO: DK 1999-1765 19991209
AN 2001-355268 [37] WPIDS
AB WO 200131989 A UPAB: 20010704
NOVELTY - Screening (M1) a library of protein variants (I) for variants with reduced antibody binding capacity, comprises generating a diversified library of (I) starting from a relevant protein backbone, transforming (L) into host cells which are cultured, sampling each cell culture, determining the antibody

binding capacity of (I), and determining the functionality of (I).

DETAILED DESCRIPTION - Screening (M1) a library of protein variants (I) for functional variants with reduced antibody binding capacity, comprises:

(i) generating a diversified library of (I) starting from a relevant protein backbone;

(ii) transforming (L) into suitable host cells which are cultured;

(iii) sampling each cell culture;

(iv) analyzing a sample by determining the antibody binding capacity of (I);

(v) analyzing a sample by determining the functionality of (I).

USE - The method is used for screening a library of protein variants for functional variants with reduced antibody binding capacity (claimed), preferably identifying protein variants with reduced immunogenecity. The method is also useful for testing compounds modified with respect to reduced allergenicity.

ADVANTAGE - The use of a diversified library of protein variants in the method is possible to screen a large number of protein variants for their ability to bind to specific antibodies in a quick and automated manner thereby providing leads that may be tested for their immunogenicity in animal studies.

Dwg.0/3

L13 ANSWER 5 OF 22 WPIDS (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: 2001-316173 [33] WPIDS
CROSS REFERENCE: 2001-245064 [25]
DOC. NO. NON-CPI: N2001-227286
DOC. NO. CPI: C2001-097349
TITLE: Removing unincorporated dye-labeled molecules from **polymer** incorporated with dye-labeled molecules, involves mixing and incubating the mixture with porous hydrophobic material entrapped within a hydrophilic matrix.
DERWENT CLASS: A96 B04 D16 P34 S03
INVENTOR(S): HUGHES, K A; KAISER, R J; MAHONEY, J E; SPICER, D A; SPRINGER, A L; STOLOWITZ, M L; WEISSMAN, C H D
PATENT ASSIGNEE(S): (PROL-N) PROLINX INC
COUNTRY COUNT: 93
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001025491	A1	20010412	(200133)*	EN	58

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

AU 2000080039 A 20010510 (200143)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001025491	A1	WO 2000-US27895	20001006

AU 2000080039 A AU 2000-80039 20001006

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000080039 A	Based on	WO 200125491

PRIORITY APPLN. INFO: US 2000-564117 20000503; US 1999-158188P 19991006; US 1999-164050P 19991108

AN 2001-316173 [33] WPIDS

CR 2001-245064 [25]

AB WO 200125491 A UPAB: 20010801

NOVELTY - Removing unincorporated dye-labeled molecules (I) from a mixture (M1) comprising (I) and a **polymer** (P) incorporated with (I), involves mixing and incubating M1 with particles that comprise a porous hydrophobic material (M2) entrapped within a hydrophilic matrix (M3), for passing unincorporated (I) into M3 and adsorbing on M2, and removing the particles and adsorbed unincorporated (I), from M1.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) preparing dye-labeled polynucleotides that are substantially free of unincorporated dye-labeled reactant, comprising:

(a) annealing a primer to a template and contacting the annealed primer with a polymerase in a reaction mixture that comprises the dye-labeled reactant, thereby extending the primer to form a number of dye-labeled polynucleotides;

(b) contacting the reaction mixture with a number of particles, where the particles have hydrophobic materials that are entrapped within a porous hydrophilic matrix, so as to effect the selective absorption of unincorporated dye-labeled reactant, and unincorporated dye-labeled artifacts derived from it; and

(c) separating the particles from the reaction mixture that contains the dye-labeled polynucleotides; and

(2) a kit for removing unincorporated (I) from M1, comprising a microtiter plate having a number of wells, where one or more of the wells contain particles that comprise M3 within which M2 is incorporated.

USE - The method is useful for removing unincorporated dye-labeled molecules from a mixture that includes dye-labeled molecules and a **polymer** into which dye-labeled molecules are incorporated (claimed).

ADVANTAGE - The method provides for the quantitative removal of all of the potentially contaminating constituents associated with DNA sequencing reactions. Extraordinary potential of capillary electrophoresis for DNA sequencing is obtained.

Dwg.0/13

L13 ANSWER 6 OF 22 WPIDS (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: 2001-245064 [25] WPIDS

CROSS REFERENCE: 2001-316173 [27]

DOC. NO. NON-CPI: N2001-174500

DOC. NO. CPI: C2001-073596

TITLE: Removing unincorporated dye-labeled molecules associated with DNA sequencing, comprises using particles made of porous hydrophobic materials

encapsulated in a hydrophilic matrix.

DERWENT CLASS: A89 B04 D16 P34 S03
INVENTOR(S): HUGHES, K A; KAISER, R J; MAHONEY, J E; SPICER, D A; SPRINGER, A L; STOLOWITZ, M L; WEISSMAN, C H D
PATENT ASSIGNEE(S): (PROL-N) PROLINX INC
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001025490	A1	20010412	(200125)*	EN	40

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2000078720 A 20010510 (200143)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001025490	A1	WO 2000-US27765	20001005
AU 2000078720	A	AU 2000-78720	20001005

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000078720	A	Based on	WO 200125490

PRIORITY APPLN. INFO: US 2000-564117 20000503; US 1999-158188P 19991006; US 1999-164050P 19991108

AN 2001-245064 [25] WPIDS
CR 2001-316173 [27]
AB WO 200125490 A UPAB: 20010801
NOVELTY - Removing (M1) unincorporated dye-labeled molecules (I) from a mixture that contains (I) and a **polymer** into which (I) are incorporated comprising:

(a) contacting the mixture with particles (II) that contain a porous hydrophobic material entrapped within a hydrophilic matrix;

(b) incubating the mixture and (II); and

(c) removing (II) and (I) adsorbed onto the hydrophobic material from the mixture, is new.

DETAILED DESCRIPTION - Removing (M1) unincorporated dye-labeled molecules (I) from a mixture that contains (I) and a **polymer** into which (I) are incorporated comprising:

(a) contacting the mixture with a number of particles (II) that contain a porous hydrophobic material entrapped within a hydrophilic matrix;

(b) incubating the mixture and (II), for (I) that are not incorporated into the **polymer** to pass into the hydrophilic matrix and become adsorbed onto the hydrophobic material; and

(c) removing (II) from the mixture and therefore removing the adsorbed (I), is new.

INDEPENDENT CLAIMS are also included for the following:

(1) preparing (M2) dye-labeled polynucleotides (III) that are substantially free of unincorporated dye-labeled reactant (IV) comprising:

(a) annealing a primer to a template and contacting the annealed primer with a polymerase in a reaction mixture that contains (IV), to extend the primer to form a number of (III);

(b) contacting the reaction mixture with (II) to effect the selective absorption of (IV) and unincorporated dye-labeled artifacts derived from (IV); and

(c) separating (II) from the reaction mixture that contains (III); and

(2) a kit for carrying out M1 comprising a microtiter plate with a number of wells, where one or more of the wells contains (II).

USE - The methods and kits are useful for removing fluorescent or other dye-labeled molecules and potentially contaminating constituents associated with DNA sequencing and for preparing dye-labeled polynucleotides that are free of unincorporated dye-labeled reactants.

ADVANTAGE - The removal of unincorporated dye-labeled molecules from the reaction products greatly reduces or eliminates artifacts that can result from the presence of unincorporated dye-labeled molecules in samples that are loaded onto e.g. gels or capillary electrophoresis columns for analysis. The methods are simple to perform, highly efficient and amenable to high-throughout, automated sequencing. A centrifugation or vacuum filtration step is nor required, both of which are difficult to adapt to an automated robotic sequencing system. In addition the use of modified e.g. biotinylated, primers is not required.

Dwg.0/12

L13 ANSWER 7 OF 22 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2002042621 MEDLINE
DOCUMENT NUMBER: 21619919 PubMed ID: 11769797
TITLE: An immunoassay for terbutryn using direct hapten **linkage** to a glutaraldehyde network on the polystyrene surface of standard microtiter plates.
AUTHOR: Holthues H; Pfeifer-Fukumura U; Hartmann I; Baumann W
CORPORATE SOURCE: Institute of Physical Chemistry, University of Mainz, Germany.
SOURCE: FRESENIUS JOURNAL OF ANALYTICAL CHEMISTRY, (2001 Dec) 371 (7) 897-902.
Journal code: 9114077. ISSN: 0937-0633.
PUB. COUNTRY: Germany: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200204
ENTRY DATE: Entered STN: 20020124
Last Updated on STN: 20020403
Entered Medline: 20020401

AB 2-Aminobutylamino-4-ethylamino-6-isopropylamino-1,3,5-triazine (ABA-atrazine) has been synthesized and used as a coating hapten in an immunoassay with a monoclonal antibody against terbutryn. Coating was achieved by covalently **linking** ABA-atrazine to a glutaraldehyde **polymer** network directly bound to the polystyrene surface of a standard **96-well** microtiter **plate**. The assay was carefully optimized. In

particular, the coating hapten concentration had a strong effect on the ELISA sensitivity. By including a pre-incubation step a low test midpoint (IC50-value) of 0.130 microg L(-1) was achieved. As far as we are aware this is the most sensitive ELISA for terbutryn yet reported. The coating-hapten-format presented is proposed as generally applicable, because the glutaraldehyde-modified microtiter **plate** surface enables stable immobilization of a broad variety of coating haptens.

L13 ANSWER 8 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:390613 BIOSIS
DOCUMENT NUMBER: PREV200100390613
TITLE: Light-induced activation of an inert surface for covalent immobilization of a protein ligand.
AUTHOR(S): Nahar, Pradip (1); Wali, Nalini Moza; Gandhi, R. P.
CORPORATE SOURCE: (1) Centre for Biochemical Technology (CSIR), Mall Road, Delhi University Campus, Delhi, 110007: p_nahar@hotmail.com India
SOURCE: Analytical Biochemistry, (July 15, 2001) Vol. 294, No. 2, pp. 148-153. print.
ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A simple and mild procedure is developed for the preparation of an activated **polymer** surface, used for immobilization of a protein ligand through a covalent **linkage**. Activation of the **polymer** surface is carried out by attaching an active functional group through 1-fluoro-2-nitro-4-azidobenzene (FNAB). UV irradiation of FNAB transforms its azido group into a highly reactive nitrene, which binds with the inert **polymer** surface, whereas the active fluoro group of FNAB, now part of the **polymer**, remains intact. Covalent **linkage** between the ligand and the inert surface is established through this active fluoro group in a thermochemical reaction. The photochemical step is carried out under dry conditions to exclude the possibility of undesirable reactions between the solvent and the highly reactive nitrene. The method can be used for activation of different inert **polymer** surfaces having carbon hydrogen bonds. The efficacy of our method is demonstrated by immobilizing horseradish peroxidase on an activated polystyrene surface. The enzyme, immobilized through the photolinker, is found to give a twofold increase in absorbance with the substrate as compared to the directly adsorbed enzyme. The method may have many applications in the preparation of bioreactors, biostrips, and biosensors, and in diagnostic tests involving the ELISA technique.

L13 ANSWER 9 OF 22 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2001:465160 SCISEARCH
THE GENUINE ARTICLE: 437YU
TITLE: Fluoresence immunoassay of alpha-fetoprotein with iron(III) tetrasulfonatophthalocyanine as a mimetic enzyme labeling reagent
AUTHOR: Yang H H; Zhu Q Z; Li D H; Lin P; Ding M T; Xu J G (Reprint)
CORPORATE SOURCE: Xiamen Univ, Dept Chem, Key Lab Analyt Sci MOE, Xiamen 361005, Peoples R China (Reprint); Xiamen Univ, Dept Mat Sci, Xiamen 361005, Peoples R China;

Xiamen Univ, Ctr Canc Res, Xiamen 361005, Peoples R China

COUNTRY OF AUTHOR: Peoples R China
SOURCE: FRESENIUS JOURNAL OF ANALYTICAL CHEMISTRY, (MAY 2001) Vol. 370, No. 1, pp. 88-91.
Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.
ISSN: 0937-0633.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 12

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A new fluorimetric immunoassay for a-fetoprotein (AFP) has been developed using a novel promising mimetic peroxidase, iron(III) tetrasulfonatophthalocyanine (FeTSPc). as a labeling reagent to catalyze the fluorescence reaction of P-hydroxyphenylacetic acid (P-HPA) and hydrogen peroxide (H2O2) In the competitive immunoassay, anti-AFP antibody was coated on a **96-well plate** (polystyrene) and a constant amount of FeTSPc-labeled AFP and a known amount of test solution were added. Non-labeled and FeTSPc-labeled AFP compete for binding to the **plate**-bound antibody. After the immunoreaction, the immunochemically adsorbed FeTSPc-AFP **conjugate** moiety was determined by measuring the fluorescence produced in a solution containing P-HPA and H2O2. AFP can be determined in the concentration range of 1-300 ng mL(-1) with a detection limit of 0.5 ng mL(-1).

L13 ANSWER 10 OF 22 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2000-618771 [59] WPIDS
DOC. NO. NON-CPI: N2000-458589
DOC. NO. CPI: C2000-185282
TITLE: Removing virus from sample involves treating sample with composition comprising solid support matrix attached to cyanovirin, its **conjugate** or **conjugate** comprising a cyanovirin coupled to anti-cyanovirin antibody.
DERWENT CLASS: A96 B04 D16 D22 P32 P34
INVENTOR(S): BOYD, M R
PATENT ASSIGNEE(S): (USSH) US DEPT HEALTH & HUMAN SERVICES
COUNTRY COUNT: 92
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000053213	A2	20000914	(200059)*	EN	93
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2000035231	A	20000928	(200067)		
EP 1162992	A2	20011219	(200206)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000053213	A2	WO 2000-US6247	20000310
AU 2000035231	A	AU 2000-35231	20000310
EP 1162992	A2	EP 2000-913869	20000310
		WO 2000-US6247	20000310

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000035231	A	Based on	WO 200053213
EP 1162992	A2	Based on	WO 200053213

PRIORITY APPLN. INFO: US 1999-416434 19991012; US 1999-267447 19990312

AN 2000-618771 [59] WPIDS

AB WO 200053213 A UPAB: 20001117

NOVELTY - A method for removing (M1) virus from a sample, is new.

DETAILED DESCRIPTION - A method for removing (M1) virus from a sample involves contacting the sample (S) with a composition (I) comprising an isolated and purified antiviral protein, peptide (both of which comprise 9 contiguous amino acids of a fully defined 101 amino acid sequence (S2) (given in the specification), that bind to the virus) and their **conjugates** which are attached to a solid support matrix, and then separating (S) and (I).

Optionally the method involves contacting the sample with (I), contacting the sample with a matrix-anchored anti-cyanovirin antibody which binds the antiviral peptide, antiviral protein, antiviral peptide **conjugate** or antiviral protein **conjugate** to which the virus is bound and separating the matrix-anchored anti-cyanovirin antibody and then separating the matrix-anchored anti-cyanovirin antibody and the sample such that the virus is removed from the sample.

INDEPENDENT CLAIMS are also included for the following:

(1) a composition (C1) comprising a solid support matrix to which is attached an isolated and purified antiviral protein, antiviral peptide (which comprise nine contiguous amino acid sequence of (S2) that binds to a virus), antiviral protein **conjugate** or antiviral peptide **conjugate**;

(2) a **conjugate** (II) comprising an isolated and purified antiviral protein or antiviral peptide comprising nine contiguous amino acids of (S2), that bind to a virus, coupled to an anti-cyanovirin antibody or one effector component, which can be the same or different groups such as polyethylene glycol, **dextran** or albumin;

(3) a composition comprising (II);

(4) a method (M2) of inhibiting prophylactically or therapeutically, a viral infection of a host, which comprises topically administering to a host an antiviral agent which is an antiviral peptide, an antiviral protein **conjugate** or a antiviral peptide **conjugate** in which antiviral protein or antiviral peptide comprises 9 contiguous amino acids of (S2) which have antiviral activity and the antiviral protein, antiviral peptide or antiviral protein or peptide **conjugate** is attached to a solid matrix, such that viral infection is inhibited; and

(5) a matrix-anchored anti-cyanovirin antibody (III), where the antibody binds to an epitope of an antiviral protein or an antiviral

peptide comprising nine contiguous amino acids of (S2), where the antibody is attached to a solid-support matrix.

ACTIVITY - Antiviral; anti-HIV. The anti-HIV activity of the cyanovirin polypeptides were tested in vitro. Cyanovirin solution were serially diluted in complete medium and added to **96-well** test **plates**. Uninfected CEM-SS cells were plated at a density of 1 x 104 cells in 50 micro l of complete medium. Diluted HIV-1 was then added to the final volume in each microtiter well was 200 micro l. Quadruplicate wells were used for virus-infected cells. **Plates** were incubated at 37 deg. C in an atmosphere containing 5% CO2 for 4, 5 or 6 days. Subsequently, aliquots of cell-free supernatant were removed from each well and analyzed for reverse transcriptase activity, p24 antigen production and synthesis of infectious virions as described. Cellular growth or viability then was estimated on the remaining contents of each well. The results showed that cyanovirin-N was capable of complete inhibition of the cytopathic effects of HIV-1 upon CEM-SS human lymphoblastoid target cells in vitro, direct cytotoxicity of the protein upon the target cells was not observed at the highest tested concentrations. Cyanovirin-N also strikingly inhibited the production of RT, p24, and SFU in HIV-1-infected CEM-SS cells within these same inhibitory effective concentrations, indicating that the protein halted viral replication.

MECHANISM OF ACTION - gp120-mediated binding and fusion of intact HIV-i virions to host cells blocker; inhibitor of cell-to-cell fusion and virus transmission.

USE - (II) and C1 are used for inhibiting prophylactically or therapeutically a viral infection of a host, such that viral infection is inhibited (claimed).

Dwg.0/12

L13 ANSWER 11 OF 22 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2000-412289 [35] WPIDS
DOC. NO. CPI: C2000-125016
TITLE: Antiviral peptides comprising a domain of 10-25 amino acids, half of which is positively charged and half uncharged, useful for treatment of human immunodeficiency virus and herpes simplex virus.
DERWENT CLASS: B04
INVENTOR(S): NIBBERING, P H; VAN, ' H W; VAN NIEUW AMERONGEN, A; VEERMAN, E C I; VANT HOF, W; VAN 'THOF, W
PATENT ASSIGNEE(S): (TEWE-N) STICHTING TECH WETENSCHAPPEN; (AMPH-N) AM-PHARMA BV
COUNTRY COUNT: 91
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000032629	A2	20000608	(200035)*	EN	18
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2000016959	A	20000619	(200044)		
NL 1010692	C2	20000606	(200044)		
EP 1147132	A2	20011024	(200171)	EN	

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000032629	A2	WO 1999-NL732	19991201
AU 2000016959	A	AU 2000-16959	19991201
NL 1010692	C2	NL 1998-1010692	19981201
EP 1147132	A2	EP 1999-960013	19991201
		WO 1999-NL732	19991201

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000016959	A	Based on	WO 200032629
EP 1147132	A2	Based on	WO 200032629

PRIORITY APPLN. INFO: NL 1998-1010692 19981201

AN 2000-412289 [35] WPIDS

AB WO 200032629 A UPAB: 20000725

NOVELTY - Antiviral peptides (I) containing a domain of 10-25 amino acids, where half the domain is positively charged and the other half is uncharged, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) oligomers (II) of (I) consisting of at least 2 (I) coupled to each other via a **spacer**; and

(2) hybrid peptide constructs comprising (I) together with another peptide, lipids, oligosaccharides, radioactive labels, organic receptor ligands and peptide **polymer conjugates**.

ACTIVITY - Antiviral; antiulcer.

In test phials 10 micro l HSV (herpes simplex virus), lab strain 96-6700 P (TCID (tissue culture effective dose)50 105-106), was supplemented with peptide and NaPB to 200 micro l. For the positive control the peptide was replaced by a human neutrophil defensin pool (HNP1-3). The test phials were subsequently incubated at 37 deg. C for 3 hours. Tenfold dilutions were then made in Dulbecco's Modified Eagles Medium (DMEM) with 2% fetal calf serum (FCS). Diverse peptides, synthesized in identical manner, were used as negative control in the experiments. Vero-cells were isolated using detachment buffer, washed and brought up to a concentration of 2x105 cells per ml DMEM + 2% FCS. One hundred micro l cell suspension was added into each well of a **96 well plate**. Acyclovir (ACV) was added as control to several wells two hours before infection. Fifty micro l of a dilution was added to each well at the point in time t=0. After 3 days of incubation in a CO2 - stove of 37 deg. C the cytopathological effect (cpe) was scored by counting under the microscope and the TCID50 was determined. In sufficiently high concentrations (50 pg/ml), peptide KRLFKKLKFSLRKY is at least as effective as acyclovir and HNP.

MECHANISM OF ACTION - None given.

USE - (I) and (II) are useful for treating viral infections such as human immunodeficiency virus (HIV) and herpes simplex virus (HSV). (I) is also useful for treating cold sores, aphthous ulcers

and viral bronchial infections.
Dwg.0/5

L13 ANSWER 12 OF 22 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2000-339654 [29] WPIDS
DOC. NO. CPI: C2000-103107
TITLE: Fusion proteins comprising interferon-beta-1a useful for inhibiting angiogenesis.
DERWENT CLASS: A96 B04 D16
INVENTOR(S): BRICKELMAIER, M; HOCHMAN, P S; RUNKEL, L; WHITTY, A; HOCHMAN, P
PATENT ASSIGNEE(S): (BIOJ) BIOGEN INC
COUNTRY COUNT: 90
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000023472	A2	20000427	(200029)*	EN	82
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW				
AU 2000013158	A	20000508	(200037)		
NO 2001001861	A	20010613	(200141)		
EP 1121382	A2	20010808	(200146)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
BR 9915548	A	20010814	(200154)		
CZ 2001001330	A3	20010912	(200158)		
KR 2001085932	A	20010907	(200218)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000023472	A2	WO 1999-US24200	19991015
AU 2000013158	A	AU 2000-13158	19991015
NO 2001001861	A	WO 1999-US24200	19991015
		NO 2001-1861	20010411
EP 1121382	A2	EP 1999-956574	19991015
		WO 1999-US24200	19991015
BR 9915548	A	BR 1999-15548	19991015
		WO 1999-US24200	19991015
CZ 2001001330	A3	WO 1999-US24200	19991015
		CZ 2001-1330	19991015
KR 2001085932	A	KR 2001-704797	20010416

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000013158	A	Based on	WO 200023472
EP 1121382	A2	Based on	WO 200023472
BR 9915548	A	Based on	WO 200023472
CZ 2001001330	A3	Based on	WO 200023472

PRIORITY APPLN. INFO: US 1999-120237P 19990216; US 1998-104491P 19981016

AN 2000-339654 [29] WPIDS

AB WO 200023472 A UPAB: 20000617

NOVELTY - Fusion proteins (I) comprising glycosylated interferon-beta (IFN- beta) (especially IFN- beta -1a), **linker** groups and non-IFN- beta proteins (especially an immunoglobulin (Ig) protein), are new.

DETAILED DESCRIPTION - An isolated polypeptide (I) comprising the amino acid sequence:

X-Y-Z (I)

X = comprises all or part of the amino acid sequence of glycosylated IFN- beta ;

Y = an optional **linker** moiety; and

Z = a polypeptide comprising all or part of the amino acid sequence of a non-IFN- beta polypeptide.

INDEPENDENT CLAIMS are included for the following:

(i) an isolated DNA sequence (II) encoding (I);

(ii) a recombinant system (III) comprising (II) operatively **linked** to an expression control sequence;

(iii) a host cell transformed with (III);

(iv) a method (IV) of producing a recombinant polypeptide, comprising:

(a) providing a population of (III);

(b) growing the cells under conditions suitable for expression of the polypeptide encoded by (II); and

(c) isolating the expressed polypeptide; and

(v) a method of inhibiting angiogenesis in a patient comprising administering (I).

ACTIVITY - Antisclerotic; antiinflammatory; immunosuppressive; cytostatic; virucide; hepatotropic; antiinflammatory; antiangiogenic.

Human venous endothelial cells (Cell Systems 2V0-P75) and human dermal microvascular endothelial cells (Cell Systems 2M1-C25) were maintained in culture with CS-C Medium Kit (RTM) (Cell Systems 4ZO-500). Twenty-four hours prior to the experiment, cells were trypsinized, and resuspended in assay medium, 90% M199 and 10% fetal bovine serum (FBS), and were adjusted to desired cell density. Cells were then plated onto gelatin-coated 24 or **96 well plates**, either at 12500 cells/well or 2000 cells/well, respectively.

After overnight incubation, the assay medium was replaced with fresh medium containing 20 ng/ml of human recombinant basic Fibroblast Growth Factor (bFGF) (Becton Dickinson 40060) and various concentrations of fusion and non-fusion IFN- beta -1a proteins or positive control (endostatin could be used as a positive control, as could an antibody to bFGF). The final volume was adjusted to 0.5 ml in the 24 well **plate** or 0.2 ml in the **96 well plate**.

After seventy-two hours, cells were trypsinized for Coulter counting, frozen for CyQuant (RTM) fluorescense reading, or labeled with (3H) thymidine. This in vitro assay tested the human IFN- beta molecules of the invention for effects on endothelial cell proliferation which may be indicative of antiangiogenic effects in vivo.

No results given.

MECHANISM OF ACTION - None disclosed.

USE - (I) may be administered to inhibit angiogenesis in a

patient (claimed). It may also be used to treat multiple sclerosis, fibrosis, other inflammatory and autoimmune diseases, cancers, hepatitis, and other diseases and viral infection characterized by neovascularization.
Dwg.0/12

L13 ANSWER 13 OF 22 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2000-317882 [27] WPIDS
DOC. NO. NON-CPI: N2000-238563
DOC. NO. CPI: C2000-096247
TITLE: Bioassay plate for detecting antigens and antibodies in immunoassays has silver ions immobilized on the plate surface.
DERWENT CLASS: A89 B04 D16 J04 P42 S03
INVENTOR(S): BONEN, M R; GARCIA, A A
PATENT ASSIGNEE(S): (UYAR-N) UNIV ARIZONA STATE
COUNTRY COUNT: 21
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000021665	A1	20000420	(200027)*	EN	37
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: CA US					
EP 1121198	A1	20010808	(200146)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000021665	A1	WO 1999-US23902	19991014
EP 1121198	A1	EP 1999-956547	19991014
		WO 1999-US23902	19991014

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 1121198	A1	Based on	WO 200021665

PRIORITY APPLN. INFO: US 1999-145786P 19990727; US 1998-104263P 19981014
AN 2000-317882 [27] WPIDS
AB WO 200021665 A UPAB: 20000606
NOVELTY - A bioassay plate with silver ions immobilized on its surface, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a method for detecting an antigen comprising:
(a) incubating a multi-well bioassay plate with silver ions immobilized on its surface with a biotinylated antibody that has specificity for the antigen to provide a plate with the antibody immobilized on its surface;
(b) incubating the plate with a solution containing the antigen;
(c) washing the plate with an aqueous solution;
(d) incubating the plate with a labeled antibody having

specificity for the antigen;
(e) washing the plate with an aqueous solution; and
(f) detecting the label, where any detection of the label is indicative of the presence of the antigen;
(2) a method for detecting a first antibody comprising:
(a) incubating a multi-well bioassay plate with silver ions immobilized on its surface with a biotinylated antigen that is reactive with the first antibody to provide a plate with the antigen immobilized on its surface;
(b) incubating the plate with an aqueous solution containing the first antibody;
(c) washing the plate with an aqueous solution;
(d) incubating the plate with an aqueous solution containing a labeled second antibody that binds to the first antibody;
(e) washing the plate with an aqueous solution; and
(f) detecting the label, where any detection of the label is indicative of the presence of the first antibody;
(3) a kit (I) for the detection of a first antibody comprising a first container containing a bioassay plate with silver ions immobilized on its surface;
(4) a kit (II) for the detection of an antigen comprising a first container containing a bioassay plate with silver ions immobilized on its surface; and
(5) an apparatus for activating microplates comprising:
(a) a housing;
(b) a reagent addition/withdrawal chamber disposed in the housing which includes reagent and wash storage containers in communication with a manifold that is in communication with dispense lines disposed to deliver wash and reagent to a microplate and further includes aspirate lines disposed to aspirate spent reagent from the microplate that are in communication with the manifold in communication with a waste container;
(c) an incubation chamber disposed in the housing which includes a device for vertically delivering a non-reactive sealing plate to the microplate and a device for heating and agitating the microplate; and
(d) a device for horizontally conveying a microplate into and out of the addition/withdrawal chamber and between the addition/withdrawal chamber and the incubation chamber.
USE - The plate is used in immunoassay systems for detecting antibodies or antigens e.g. enzyme **linked** immunosorbent assays. The apparatus provides an automated process for using the plates.
ADVANTAGE - The silver coated plates are more sensitive than the streptavidin-coated plates used previously.
Dwg.0/11

L13 ANSWER 14 OF 22 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2000-138978 [13] WPIDS
CROSS REFERENCE: 1999-340250 [29]
DOC. NO. NON-CPI: N2000-103973
DOC. NO. CPI: C2000-042937
TITLE: Ionisation of analytes, particularly biopolymers, by matrix assisted pulsed laser desorption uses ether polyol derivative as liquid matrix substance on carrier plate.
DERWENT CLASS: A35 A96 B04 D16 S03 V05
INVENTOR(S): FRANZEN, J; KOESTER, C

PATENT ASSIGNEE(S): (BRUK-N) BRUKER DALTONIK GMBH
COUNTRY COUNT: 2
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
GB 2340298	A	20000216	(200013)*		22
DE 19834070	A1	20000210	(200015)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
GB 2340298	A	GB 1999-17513	19990726
DE 19834070	A1	DE 1998-19834070	19980729

PRIORITY APPLN. INFO: DE 1998-19834070 19980729
AN 2000-138978 [13] WPIDS
CR 1999-340250 [29]
AB GB 2340298 A UPAB: 20000313
NOVELTY - Ionisation of an analyte substance by matrix assisted pulsed laser desorption (**MALDI**) using a liquid matrix substance on a carrier **plate**, is new. The liquid matrix comprises an ether polyol containing at least 3 hydroxyl groups and having at least one ether bond in the carbon chain.

USE - The method is used for the ionisation of high-molecular weight analyte molecules, particularly of large biopolymers, which is employed for mass-spectrometric analysis of ions, particularly for determination of their molecular weight. Biomolecules which can be investigated are oligonucleotides, proteins and polysaccharides, including their analogues and **conjugates** e.g. glycoproteins or lipoproteins, and artificially produced **polymers**.

ADVANTAGE - The method provides a highly sensitive method for gentle, pulsed ionisation of very large molecules which is capable of automation without fragment or adduct formation. Many samples may be applied simultaneously. The liquid matrix substances used have very low vapour pressures (lower than the vapour pressure of glycerol currently used) and are suitable for energy absorption and ionisation by infrared lasers (IR-MALDI), as well as other lasers when additional light-absorbing compounds are included. The liquid matrix substances are miscible with water and can dissolve practically all analyte molecules if introduced in sufficiently low concentrations.

Dwg.0/4

L13 ANSWER 15 OF 22 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000227435 MEDLINE
DOCUMENT NUMBER: 20227435 PubMed ID: 10766368
TITLE: Determination of MAG-camptothecin, a new **polymer**-bound camptothecin derivative, and free camptothecin in dog plasma by HPLC with fluorimetric detection.
AUTHOR: Fraier D; Frigerio E; Brianceschi G; Casati M; Benecchi A; James C
CORPORATE SOURCE: Pharmacia and Upjohn, Drug Metabolism Research, Pharmacokinetics and Metabolism Department, Milan,

Italy.
SOURCE: JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS, (2000 Apr) 22 (3) 505-14.
Journal code: A2C; 8309336. ISSN: 0731-7085.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000811
Last Updated on STN: 20000811
Entered Medline: 20000803

AB A high throughput. selective and sensitive high-performance liquid chromatographic (HPLC) method for the determination of a water-soluble **polymer**-bound Camptothecin **conjugate** (MAG-CPT) and Camptothecin (CPT) in dog plasma has been developed and validated. The method involved the analysis of free and total CPT (free + **polymer**-bound). Free CPT (intact lactone plus carboxylate) was extracted from acidified plasma using Oasis SPE material in **96-well plates**. For the assay of the total CPT, plasma proteins were first precipitated with methanol in a **96-well plate** containing a 10-microm melt blown polypropylene membrane. The methanolic supernatant was separated and collected into a second **96-well plate** by simply applying vacuum to the **plate**. After hydrolysis at pH 9.8 for 18 h and re-acidification, samples were injected directly from the collection **plate** onto the HPLC system. MAG-CPT concentration was then calculated by subtraction of free from total CPT. The LLOQs of the method were 1.17 ng/ml for free CPT and 103.10 ng/ml (as CPT equivalent) for MAG-CPT using 0.1 and 0.05 ml of plasma, respectively. Linearity, precision, accuracy and recovery of the method were evaluated. The stability of MAG-CPT in plasma alone and after its stabilisation was carefully evaluated. No interference from blank dog, mouse and human plasma was observed. The suitability of the method for in vivo samples was assessed by the analysis of samples obtained from dogs that had received a single and 5-day repeated dose of MAG-CPT.

L13 ANSWER 16 OF 22 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1999-216694 [19] WPIDS
DOC. NO. CPI: C1999-063950
TITLE: Increasing the shelf life or stability of a coated substrate.
DERWENT CLASS: A23 A96 B04 D16 P34
INVENTOR(S): ILSLEY, S R; MANNUZZA, F J; MYLES, A; SWIDEREK, M S
PATENT ASSIGNEE(S): (BECT) BECTON DICKINSON & CO
COUNTRY COUNT: 27
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 905231	A2	19990331	(199919)*	EN	9
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
JP 11164685	A	19990622	(199935)		29
US 5932473	A	19990803	(199937)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 905231	A2	EP 1998-307526	19980916
JP 11164685	A	JP 1998-278645	19980930
US 5932473	A	US 1997-941473	19970930

PRIORITY APPLN. INFO: US 1997-941473 19970930
AN 1999-216694 [19] WPIDS
AB EP 905231 A UPAB: 20011203
NOVELTY - A method for increasing the shelf life or stability of a coated substrate is new and comprises applying the coating to the substrate.

DETAILED DESCRIPTION - Applying a coating to a substrate comprises:

(a) coating a substrate with about 5-1000 mu g/ml of a cell adhesion promoter (I) in an about 0.005-0.5 M salt solution;

(b) incubating (I) on the substrate; and

(c) rinsing and drying the coated substrate.

An INDEPENDENT CLAIM is also included for an in vitro culturing system comprising a substrate, and a coating of about 5-1000 mu g/ml of a cell adhesion promoter (I) in an about 0.005-0.5 M salt solution.

USE - The method is used to improve the shelf-life of coated substrates. Such substrates are used to promote adhesion, attachment and growth of e.g. epithelial cells, fibroblasts, immunocompetent cells and thymocytes. The cells may be used for study of individual and interactive cell metabolism, the effect of infectious agents, genetic composition, production of specific compounds such as DNA and RNA and the re-implantation of cells for skin, corneal grafts, brain, vascular grafts and in vitro fertilization.

ADVANTAGE - The stability of poly-D-**lysine** coated surfaces is dependent on the counter anion used in the coating process e.g. when phosphate buffered saline (PBS) is used as the solution in which to apply the coating to the substrate the shelf life is then dependent on the type of substrate e.g. dish or flask, and on the storage temperature, humidity and packaging. The new method involves applying the coating in a solution which has citrate or sulfate as the counter anion. The shelf-life is then lengthened two- or threefold, is independent of the packaging and the substrates can be stored at room temperature.

Dwg.0/0

L13 ANSWER 17 OF 22 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999414096 MEDLINE
DOCUMENT NUMBER: 99414096 PubMed ID: 10482898
TITLE: Direct **matrix**-assisted **laser** desorption/**ionization** mass spectrometric analysis of glycosphingolipids on thin layer chromatographic **plates** and transfer membranes.
AUTHOR: Guittard J; Hronowski X L; Costello C E
CORPORATE SOURCE: Mass Spectrometry Resource, Departments of Biochemistry and Biophysics, Boston University School of Medicine, Boston, MA 02118-2526, USA.
CONTRACT NUMBER: P41 RR 10888 (NCRR)

SOURCE: RAPID COMMUNICATIONS IN MASS SPECTROMETRY, (1999) 13 (18) 1838-49.
Journal code: A9Q; 8802365. ISSN: 0951-4198.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991026

AB Results are reported for analysis by **matrix**-assisted **laser** desorption/**ionization** time-of-flight mass spectrometry (**MALDI**-TOFMS) of native glycosphingolipids (GSLs) after development on thin layer chromatographic **plates** and after heat transfer of the GSLs from the **plates** to several types of **polymer** membranes. The spectral quality is better for membrane-bound analytes, in terms of sensitivity, mass resolution and background interference. The sensitivity gain compared with liquid secondary ion mass spectrometry (LSIMS) of GSLs on thin layer **plates** is 1-2 orders of magnitude (detection limits of 5-50 pmol vs. 1-10 nmol). Resolution and mass accuracy (0.1%) are limited by the irregular membrane surfaces and this effect cannot be entirely compensated by delayed extraction. The best results were obtained with a polyvinylidene difluoride (PVDF) P membrane, with irradiation from a nitrogen laser. Although the Nafion membrane could not be used for molecular weight profiling, its acidic character led to sample hydrolysis at the glycosidic **linkages**, thus yielding a series of fragments that could be used to determine the sequence of carbohydrate residues. Structural information could also be obtained by post-source decay (PSD) experiments on mass-selected precursor ions. Samples containing both neutral and acidic components were characterized in a 1:1 combination of 2, 5-dihydroxybenzoic acid and 2-amino-5-nitropyridine. GSLs that exhibited binding to antibodies in an overlay assay on the TLC **plate** were transferred to membranes and analyzed by **MALDI**-TOFMS without interference from the antibody or the salts and buffers used during the binding and visualization steps. Taking advantage of the insights into sample preparation gained from these studies, future research will extend this approach to analysis by **matrix**-assisted **laser** desorption/**ionization** Fourier transform ion cyclotron resonance mass spectrometry (**MALDI**-FTICRMS) with an external ion source.

L13 ANSWER 18 OF 22 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 1998:9471 SCISEARCH
THE GENUINE ARTICLE: YL604
TITLE: From cytotoxicity to biocompatibility testing in vitro: cell adhesion molecule expression defines a new set of parameters
AUTHOR: VanKooten T G (Reprint); Klein C L; Kohler H; Kirkpatrick C J; Williams D F; Eloy R
CORPORATE SOURCE: UNIV MAINZ, INST PATHOL, LANGENBECKSTR 1, D-55101 MAINZ, GERMANY (Reprint); UNIV LIVERPOOL, DEPT CLIN ENGN, LIVERPOOL L69 3BX, MERSEYSIDE, ENGLAND; BIOMATECH SA, F-38670 CHASSE SUR RHONE, FRANCE

COUNTRY OF AUTHOR: GERMANY; ENGLAND; FRANCE
SOURCE: JOURNAL OF MATERIALS SCIENCE-MATERIALS IN MEDICINE, (DEC 1997) Vol. 8, No. 12, pp. 835-841.
Publisher: CHAPMAN HALL LTD, 2-6 BOUNDARY ROW, LONDON, ENGLAND SE1 8HN.
ISSN: 0957-4530.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: ENGI
LANGUAGE: English
REFERENCE COUNT: 11

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Determination of potential cytotoxicity is a central issue in current biocompatibility testing standards such as ISO and ASTM. Most of these tests do not assess biocompatibility of a biomaterial with regard to cell function. This study was aimed at screening a number of potential parameters that could be included in assessment of cell functional aspects of biocompatibility. Human umbilical vein endothelial cells (HUVEC) were seeded directly on titanium, NiCr alloy, CoCr alloy, PMMA, PE, PU, PVC, and silicone, or were exposed to the material extracts. Cytotoxicity was assessed for these materials through MTT conversion, crystal violet protein determination and Ki67 expression. In addition, expression of the cell adhesion molecules E-selectin, cadherin-5 and PECAM, as well as of the adhesion associated proteins fibronectin and vinculin (focal adhesions), was determined by immunocytochemistry and western blotting. Cytotoxicity was not detected with the material extracts. Cells were able to adhere to bare metals, but not **polymers** . Fibronectin preadsorption resulted in adhesion and spreading also on the **polymers**. Cells were able to establish cell-cell contacts and focal adhesions. Western blotting, in combination with differential detergent extraction, indicated that **linkage** of cell-cell adhesion markers to the cytoskeleton may be used as an additional parameter relevant to cell function.

L13 ANSWER 19 OF 22 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 94312394 MEDLINE
DOCUMENT NUMBER: 94312394 PubMed ID: 8038171
TITLE: Interactions of microtubule-associated protein MAP2 with unpolymerized and polymerized tubulin and actin using a **96-well** microtiter **plate** solid-phase immunoassay.
AUTHOR: Pedrotti B; Colombo R; Islam K
CORPORATE SOURCE: Department of Biology, University of Milan, Italy.
SOURCE: BIOCHEMISTRY, (1994 Jul 26) 33 (29) 8798-806.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940905
Last Updated on STN: 19950307
Entered Medline: 19940825

AB A solid-phase immunoassay is used to study the protein-protein interactions between microtubule-associated protein MAP2 and the cytoskeletal proteins tubulin and actin. The assay can be performed on **96-well** microtiter **plates** and can be used to study the interactions with both subunit proteins and

their respective **polymers**, microtubules and microfilaments. The microtiter format allows a large number of samples to be processed, and a number of conditions can be varied. In this solid-phase immunoassay MAP2 bound to microtubules/microfilaments and tubulin dimers/G-actin in a concentration-dependent manner. However, the bound MAP2 was not dissociated from the filaments even at high NaCl concentrations, while simultaneous addition of NaCl diminished MAP2 binding to these proteins. MgCl2 was 1 order of magnitude more efficient in decreasing MAP2 binding compared with NaCl, suggesting that MAP2 may act by "screening" the electrostatic repulsion between tubulin dimers. The role of MAP2 in cross-**linking** microfilaments and microtubules was also examined. Microtubule/tubulin-bound MAP2 showed a diminished ability to bind to both microfilaments and G-actin, while microfilament/G-actin-bound MAP2 was able to bind efficiently to both microtubules and tubulin dimers.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 20 OF 22 JICST-EPlus COPYRIGHT 2002 JST
ACCESSION NUMBER: 910180001 JICST-EPlus
TITLE: Biochemistry and cell biology of cell adhesion proteins from domestic animals. Binding of serum vitronectin to collagen.
AUTHOR: HAYASHI MASAO; ISHIKAWA MICHIKO
CORPORATE SOURCE: Ochanomizu Univ., Faculty of Science
SOURCE: Shokuniku ni kansuru Josei Kenkyu Chosa Seika Hokokusho, (1990) vol. 8(1989), pp. 366-371. Journal Code: X0296A (Fig. 3, Tbl. 1, Ref. 9)
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
LANGUAGE: Japanese
STATUS: New
AB The present study describes that the collagen-binding activity of vitronectin in human serum increases by treatments with heparin and urea. Vitronectin purified from human serum is known to bind to native collagen, whereas vitronectin in the serum could not bind. We have examined this discrepancy. The amount of vitronectin bound to type I collagen immobilized on **96-well** microtiter **plates** was measured by ELISA using horseradish peroxidase-**conjugated** anti-vitronectin. Vitronectin in human serum considerably bound to collagen when the serum was boiled in 8M urea for 5min and mixed with heparin. Each treatment of urea, boiling, or heparin alone inefficiently activated the binding. The effective concentrations were 2M to 8M of urea and 0.05 to 1.5.MU.g/ml of heparin. **Dextran** sulfate and dermatan sulfate could substitute for heparin, but keratan sulfate, chondroitin sulfate A and C, heparan sulfate, and hyaluronate could not. (author abst.)

L13 ANSWER 21 OF 22 JICST-EPlus COPYRIGHT 2002 JST
ACCESSION NUMBER: 890188887 JICST-EPlus
TITLE: Immunohistochemical study of periodontal disease. ELISA analysis of prostaglandins and prostaglandin antibodies.
AUTHOR: MIURA KATSUTOSHI
CORPORATE SOURCE: Osaka Dental Univ.
SOURCE: Shika Igaku (Journal of the Osaka Odontological Society), (1988) vol. 51, no. 6, pp. 1088-1100.

Journal Code: G0582A (Fig. 11, Tbl. 2, Ref. 28)
CODEN: SIGAAE; ISSN: 0030-6150
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
LANGUAGE: Japanese
STATUS: New

AB The condition and extent of localization of prostaglandins(PG) in human gingiva with periodontal disease and the application of HPLC and GC-MS in this field were examined as part of an immunohistochemical study of periodontal disease. The aim of this study was to establish the enzyme-**linked** immunosorbent assay(ELISA) method of PG and PG antibodies. Reaction of PGE2 (2mg) with carrier molecules (10mg each, BSA, HSA or PL) in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride(CDI) (5mg) in phosphate buffer (pH5.5) gava a PG-**conjugate** with molar ratio of PG to carrier of approximately 10:1. A polyvinyl chloride **96 well plate** was used to adsorb the antigen of the PG-PL **conjugate**. Adsorption of **96 well plate** surface was saturated with a PG-PL concentration of 1.MU.g/ml overnight at 4.DEG.C. Adsorption increased in proportion to the concentration of PG-PL in the range of 0.1-1.0.MU.g/ml. Optical density at the saturated adsorption antigen gave sigmoid curves in the range of 1,600-25,600 dilutions for the first antibody and 400-12,800 dilutions for the second antibody. Standard curves of PG were obtained by competition of the PG-PL on the **plate** with free PGE2 (1pg-1.MU.g). The titer of rabbit No. 9 anti PG serum by ELISA, which means maximum dilutions having absorbance of 0.1 at 405nm, was 25,600. Low limits for detection by immunohistochemical methods, (fluorescence antibody technique(FITC), immunoperoxidase method(PER), peroxidase-antiperoxidase method(PAP)) and ELISA for rabbit No. 9 anti PG serum were 50, 0, 800 and 25,600 dilutions, respectively.(abridged author abst.)

L13 ANSWER 22 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1981:147160 BIOSIS
DOCUMENT NUMBER: BA71:17152
TITLE: SCREENING AND REPLICA PLATING OF ANTI HAPTEN HYBRIDOMAS WITH A TRANSFER TEMPLATE HEMOLYTIC SPOT ASSAY.
AUTHOR(S): BANKERT R B; DESOYE D; POWERS L
CORPORATE SOURCE: DEP. IMMUNOL. RES., ROSWELL PARK MEML. INST., BUFFALO, N.Y. 14263, USA.
SOURCE: J IMMUNOL METHODS, (1980) 35 (1-2), 23-32.
CODEN: JIMMBG. ISSN: 0022-1759.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB A localized hemolysis in gel assay is described for screening microcultures of hybridomas for the production of anti-hapten antibody. The keys to the rapid screening assay are a special fenestrated transfer template and an improved hapten [4-aminophthalate] **conjugated** target cell [**dextran** -coupled sheep red blood cells]. The transfer template is a **96-well plate** with a calibrated hole in the bottom center of each well. To assay for anti-hapten antibodies, the transfer template is positioned over a **96-well** microculture **plate** containing the growing hybridomas. After making contact with the tissue culture supernatant each

orifice of the transfer template retains approximately 2 .mu.l of tissue culture supernatant. The transfer template is then placed onto an assay slide containing athin layer of hapten **conjugated** target erythrocytes incorporated into agarose. After incubation with an anti-Ig and complement, areas of localized hemolysis in the gel indicate hybridomas which are secreting anti-hapten antibodies. The assay detects as little as 10 pg of antibody. Since the transfer template can be used as a replica **plate**, samples can be repeatedly transfered to various slides which contain different hapten target cells or different hapten analog inhibitors in the agarose layer. In addition of rapdily screening microcultures for positive hybridomas, this procedure permits the characterization of each monoclonal antibody's fine specificity.

FILE 'CAPLUS' ENTERED AT 11:56:04 ON 24 APR 2002
L14 2 S L4 AND CROSSLINK?
L15 2 S L14 NOT L11

L15 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:613437 CAPLUS
DOCUMENT NUMBER: 134:157043
TITLE: Use of reaction scavengers to speed synthesis and bioactivity screening in drug development
AUTHOR(S): Szafranski, Cory A.
CORPORATE SOURCE: Agilent Technologies, Wilmington, DE, 19808, USA
SOURCE: American Biotechnology Laboratory (2000), 18(9), 14, 16
CODEN: ABLAEY; ISSN: 0749-3223
PUBLISHER: International Scientific Communications, Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review without refs. is given. To properly evaluate the bioactivity of combinatorial libraries, successive steps in the synthetic scheme must be executed with high yield while minimizing losses and contamination. At each stage, rigorous postreaction purifn. helps remove excess reagents and unwanted byproducts that could reduce the yield of successive intermediates or interfere with both intermediate and final product evaluation. CombiZorb solid-phase scavengers (Agilent Technologies, Wilmington, DE) overcome the problems encountered when using ordinary **polymer**-bound reagents to purify reaction mixts. commonly found in parallel synthesis. Other high-throughput synthesis applications for the scavengers include medicinal chem. and other soln.-based reactions that require efficient postreaction purifn. In addn., the scavengers provide a rapid purifn. alternative to time-consuming liq.-liq. extns. and liq. chromatog. sepns. CombiZorb scavengers are functionally modified adsorbents - reactive silica or functionalized macro-porous polystyrene **crosslinked** with divinylbenzene (PS/DVB). The use of both silica- and org. **polymer**-based matrixes makes possible the cost-effective development of a selection of adsorbents designed to scavenge the reagents commonly employed in combinatorial synthesis and to operate under a broad range of reaction conditions, including solvents, pH, and temp. Compared with conventional gel-type **polymer** products, CombiZorb adsorbents exhibit minimal (PS/DVB-based) to no (silica-based) solvent swelling, and this facilitates both ease and flexibility of use as well as rapid

purifn. reactions. The scavengers may be added in bulk to reaction mixts. or to reactants in **96-well plates** , or may be used in flow-through applications packed in the wells of **96-well plates**, columns, or cartridges. The low swelling characteristic imparts significant scavenging power in a relatively small vol. (low diln. factor), enabling rapid and efficient removal of reagents and/or byproducts with minimal handling losses.

L15 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:476562 CAPLUS
DOCUMENT NUMBER: 121:76562
TITLE: Interactions of Microtubule-Associated Protein MAP2 with Unpolymerized and Polymerized Tubulin and Actin Using a **96-Well** Microtiter **Plate** Solid-Phase Immunoassay
AUTHOR(S): Pedrotti, Barbara; Colombo, Roberto; Islam, Khalid
CORPORATE SOURCE: Department of Biology, University of Milan, Milan, Italy
SOURCE: Biochemistry (1994), 33(29), 8798-806
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A solid-phase immunoassay is used to study protein-protein interactions between microtubule-assocd. protein MAP2 and the cytoskeletal proteins, tubulin and actin. The assay can be performed on **96-well** microtiter **plates** and can be used to study the interactions with both subunit proteins and their resp. **polymers**, microtubules and microfilaments. The microtiter format allows a large no. of samples to be processed, and a no. of conditions can be varied. In this solid-phase immunoassay, MAP2 bound to microtubules/microfilaments and tubulin dimers/G-actin in a concn.-dependent manner. However, the bound MAP2 was not dissocd. from the filaments even at high NaCl concns., whereas simultaneous addn. of NaCl diminished MAP2 binding to these proteins. MgCl2 was 1 order of magnitude more efficient in decreasing MAP2 binding compared with NaCl, suggesting that MAP2 may act by screening the electrostatic repulsion between tubulin dimers. The role of MAP2 in **crosslinking** microfilaments and microtubules was also examd. Microtubule/tubulin-bound MAP2 showed a diminished ability to bind to both microfilaments and G-actin, while microfilament/G-actin-bound MAP2 was able to bind efficiently to both microtubules and tubulin dimers. These differences in MAP2 behavior, depending on the initial binding partner, may be physiol. important in the cellular coordination of filament distribution. Although the solid-phase assay was used to study MAP2 interactions, it is felt that the assay could be generally applied to other MAPs.

[redacted]NE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, [redacted]JAPIO' ENTERED AT 11:56:39 ON 24 APR 2002)
L16 0 S L14

[redacted] ENTERED AT 11:57:27 ON 24 APR 2002)
L17 15 SEA ABB=ON PLU=ON L3 AND ((PROTEIN OR POLYPROTEIN OR PEPTIDE OR POLYPEPTIDE)(5A)IMMOBIL?)
L18 15 SEA ABB=ON PLU=ON L17 NOT (L11 OR L14)

L18 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:869656 CAPLUS
TITLE: Immunofluorscense - still the "gold standard" in ANA testing?
AUTHOR(S): Kumagai, Shunichi; Hayashi, Nobuhide
CORPORATE SOURCE: Department of Clinical and Laboratory Medicine, Kobe University School of Medicine, Hyogo, 650-0017, Japan
SOURCE: Scand. J. Clin. Lab. Invest., Suppl. (2001), 61(235), 77-83
CODEN: SCLSAH; ISSN: 0085-591X
PUBLISHER: Taylor & Francis
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A usefulness of enzyme immunoassay (EIA)-based antinuclear antibodies (ANA) tests was evaluated in comparison with the immunofluorescence ANA assay (IF-ANA). COBAS-ANA and MBL-ANA were used, in the former a mixt. of antigens extd. from HEp-2 cells and multiple recombinant antigens was immobilized on beads as the antigen, and in the latter 9 kinds of purified or recombinant **proteins** are **immobilizedon 96-well plates**. We first compared an ability to differentiate 258 connective tissue disease (CTD) patients (except rheumatoid arthritis) from 257 healthy subjects between COBAS-ANA and IF-ANA. The sensitivity and specificity of COBAS-ANA were 84 % and 94 %, resp., while those of IF-ANA at a cutoff diln. of 1:160 were 81 % and 87 %. The receiver operating characteristic (ROC) anal. showed a significant superiority of COBAS-ANA to IF-ANA. Moreover, when the cutoff index was set at 0.6, the COBAS-ANA could detect the 8 disease-specific ANAs as well as IF-ANA at a cutoff diln. of 1:40. A possible availability of MBL-ANA in a periodic health examn. in certain towns was also demonstrated. Among the 1123 subjects, a total of 145 disease-specific ANAs were detected in 126 subjects. MBL-ANA could catch disease-specific ANAs with almost same efficacy of IF-ANA. Annual survey of the residents by MBL-ANA may lead to a detection of CTD patients. EIA-based ANA tests are very useful for both detecting disease-specific ANAs and screening CTD patients. We believe that EIA-ANA should be the "gold std. esp. for screening a large no. of samples, although there is some room for tech. improvement.
REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:720015 CAPLUS
DOCUMENT NUMBER: 136:106397
TITLE: Simple dip strip ELISA for airborne estrogenic steroids
AUTHOR(S): Armstrong, S.; Miao, Z.-F.; Rowell, F. J.; Ali, Z.
CORPORATE SOURCE: School of Sciences, Institute of Pharmacy and Chemistry, North East Biotechnology Centre, University of Sunderland, Sunderland, SR1 3SD, UK
SOURCE: Analytica Chimica Acta (2001), 444(1), 79-86
CODEN: ACACAM; ISSN: 0003-2670

PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An ELISA for the potent estrogenic steroids, ethenyl estradiol (ETED), 17-.beta.-estradiol (ED), and estrone (ES), was developed and used to det. the recovery of ED and ETED following spiking from 7 filters commonly used in samplers to ascertain worker exposure to airborne biochems. Best results were obtained with cellulose nitrate (CN) and polytetrafluoroethylene (PTFE) filters. Assay reagents were also used to develop a simple dip strip assay which can be used to det. the presence of steroids on the filters. Steroids captured from air on the filter surface within a conventional personal exposure sampler were extd. with specific antiserum. A spot of hapten-**protein** conjugate is **immobilized** on a small square of CN filter attached to a plastic strip. This is immersed in the sampler soln. where unbound antibodies bind to hapten on the spot. Strips are then transferred to a **96 well** filter **plate** located within a filter manifold. Following washing, strips are incubated with alk. phosphatase-labeled second antibody and spots are developed by adding substrate. The intensity of the developed blue spot is inversely proportional to the amt. of steroid originally captured on the filter. A batch of >50 samplers can be screened within 1 h. Spots on strips from filters on which 10 ng ED, ES, or ETED is present can be visually discerned from strips from filters where no steroid is present.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:499782 CAPLUS
DOCUMENT NUMBER: 135:87147
TITLE: Ex-vivo test kit for testing the effectiveness of reversers of multidrug resistance
INVENTOR(S): Stein, Wilfred Donald; Kott, Miriam Viviana
PATENT ASSIGNEE(S): M.D.R. Test Ltd, Israel
SOURCE: U.S., 6 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6258526	B1	20010710	US 2000-487429	20000119
EP 1122541	A2	20010808	EP 2001-300397	20010117
EP 1122541	A3	20010816		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001281238	A2	20011010	JP 2001-10949	20010119

PRIORITY APPLN. INFO.: US 2000-487429 A 20000119
AB The invention provides an ex-vivo test kit for testing the effectiveness of reversers of multidrug resistance in the blood, serum or plasma of a patient contg. the reversers, the kit comprising a plurality of cell membranes from highly drug-resistant cells, the cell membranes contg. proteins which pump drugs and the

membranes being resp. attached to a plurality of support surfaces, and a dye which provides a light signal, which dye is pumped by the proteins contained in the membranes. Cell membranes from CHO CR1R12 cells were attached to polylysine-coated wells of **96-well** culture **plates**. The uptake of rhodamine to the attached membranes was measured using ATP and MgSO4 to activate the protein pump. Rhodamine was liberated from the membrane using octylglucoside.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:855273 CAPLUS
DOCUMENT NUMBER: 135:103059
TITLE: Protein-ribosome-mRNA display: affinity isolation of enzyme-ribosome-mRNA complexes and cDNA cloning in a single-tube reaction
AUTHOR(S): Bieberich, Erhard; Kapitonov, Dmitri; Tencomnao, Tewin; Yu, Robert K.
CORPORATE SOURCE: Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA, 30912, USA
SOURCE: Analytical Biochemistry (2000), 287(2), 294-298
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An enzyme-ribosome-mRNA complex was specifically purified by binding to the immobilized enzyme substrate and the cDNA was cloned in a single-tube reaction by one-step reverse transcription-PCR. The ganglioside GM3, used by sialyltransferase II (ST-II) as a substrate, was coated on a **96-well** microtiter **plate** and ST-II was in vitro transcribed and translated from a cDNA library. The isolation of an enzyme-specific protein-ribosome (PRIME) complex was achieved with as little as 0.1 ng ST-II-specific cDNA in 5 .mu.g of a total plasmid prepn. or with the cDNA prepd. from sublibraries previously inoculated at a d. of 2000 clones/culture well. The affinity purifn. of the PRIME complex was highly specific for GM3 and did not result in cDNA amplification when a different ganglioside (GM1) was used for coating of the microtiter plate. The amplified cDNA was used for cloning or a second round of ribosome display, providing a fast anal. of enzyme affinity to multiple substrates. PRIME display can be used for host-free cDNA cloning from mRNA or cDNA libraries and for binding site mapping of the in vitro translated protein. The use of a single-tube reaction in ligand-coated microtiter plates indicates the versatility of PRIME display for cDNA cloning by automated procedures. (c) 2000 Academic Press.

REFERENCE COUNT: 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:719211 CAPLUS
DOCUMENT NUMBER: 134:40730
TITLE: A novel high-throughput method for accurate, rapid, and economical measurement of multiple

Type 1 diabetes autoantibodies

AUTHOR(S): Woo, W.; LaGasse, J. M.; Zhou, Z.; Patel, R.; Palmer, J. P.; Campus, H.; Hagopian, W. A.
CORPORATE SOURCE: Department of Medicine, University of Washington, Seattle, WA, 98195, USA

SOURCE: Journal of Immunological Methods (2000), 244(1-2), 91-103
CODEN: JIMMBG; ISSN: 0022-1759
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Prediction of Type 1 diabetes for study of preventive therapies requires screening the general population, where 85% of new cases occur. Even with HLA-based prescreening, nearly 20% of all children will need multiple serum autoantibody testings. High-throughput, economical, and accurate methods are therefore essential. The authors have developed such a radiobinding method, using **96-well** microtiter **plates** and a novel immune complex capture method via membrane-bound Protein A. Each microtiter plate contained a std. neg. control serum, and low-, medium-, and high-level pos. control sera. All sera were evaluated in triplicate. This readily allowed quality control criteria both for triplicates of individual sera and for each **96-well plate**. Inter-assay coeffs. of variation (CVs) were all .ltoreq.16%, while intra-assay CVs were all .ltoreq.10%. The assay was sensitive (to detect autoantibodies in patients) and specific (low reactivity in thousands of healthy volunteers). The format worked well using diverse antigens such as 35S-met-GAD65, 35S-met-ICA512/IA2, 35S-met-Phogrin, and 125I-insulin, and could be used for simultaneous screening of reactivity to both GAD65 and ICA512/IA2 in the same well. Diagnostic accuracy compared favorably with microcentrifuge tube-based Protein A-agarose GAD65 and IA2 autoantibody radiobinding assays and with acid-charcoal-polyethylene glycol (PEG) based competitive insulin autoantibody assays. In the case of 125I-insulin, comparing signal in the absence vs. presence of cold insulin competitor was not necessary. Total serum vols. required were only 6 .mu.l for GAD and ICA512, and only 15 .mu.l for IAA. The method costs less than all other commonly used formats, and should be useful for population screening.

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:579875 CAPLUS
DOCUMENT NUMBER: 134:290806

TITLE: The use of **immobilized** mismatch binding **protein** in mutation/SNP detection

AUTHOR(S): Wagner, Robert; Dean, Alan
CORPORATE SOURCE: Gene Check Inc., Fort Collins, CO, USA

SOURCE: Methods in Molecular Biology (Totowa, New Jersey) (2000), 152(DNA Repair Protocols), 159-168
CODEN: MMBIED; ISSN: 1064-3745
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal

LANGUAGE: English

AB Protocols are given for the **immobilized** mismatch binding **protein** (IMBP) mutation/single-nucleotide polymorphism detection system. The IMBP mutation detection technol. is based on MutS, the mismatch recognition and binding component of the Escherichia coli mismatch repair system. Biotin-labeled probes are annealed to excess unlabeled PCR products amplified from genomic DNA. The hybridized fragments are added to **96-well** microtiter **plates** contg. immobilized MutS. Test fragments that are not identical to the probe sequence will anneal to form duplexes with mismatches which will be retained by the immobilized MutS.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:454281 CAPLUS

DOCUMENT NUMBER: 131:85159

TITLE: Use of mass fingerprinting for identification of protein affinity ligands

INVENTOR(S): Pennington, Stephen Roy

PATENT ASSIGNEE(S): The University of Liverpool, UK

SOURCE: PCT Int. Appl., 42 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9935502	A1	19990715	WO 1999-GB71	19990108
W: JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1046038	A1	20001025	EP 1999-900568	19990108
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, IE				
JP 2002501176	T2	20020115	JP 2000-527833	19990108

PRIORITY APPLN. INFO.: GB 1998-378 A 19980108
WO 1999-GB71 W 19990108

AB The present invention termed "inverse screening" provides a method to identify and isolate mols. that specifically and selectively recognize proteins - these mols. being called "protein affinity ligands". The method enables "protein affinity ligands" to be obtained not only for proteins of known identity but also those where prior knowledge or access to the individual proteins for which the "protein affinity ligands" are isolated were not available. Moreover, the method can be used in a targeted process whereby "protein affinity ligands" for a protein of interest - that may or may not be available in purified form and may or may not be of previously known identity - are generated and identified. Alternatively, the method can be used in a shotgun process whereby "protein affinity ligands" to multiple proteins are generated and isolated simultaneously. Again, this may be achieved without requiring access to the individual proteins or indeed prior knowledge of their identity. The inverse screening method was used to screen a polyclonal antibody and monoclonal antibodies to bovine

serum albumin (BSA) against a mixt. of proteins contg. BSA. The antibodies were immobilized on PVDF membranes in the wells of **96-well** filtration **plates**, ficoll block was applied, and the antibodies were exposed to the protein mixt. Bound protein was eluted by incubation with formic acid soln. Eluted proteins were analyzed by peptide mass fingerprinting (tryptic digestion and anal. by MALDI mass spectrometry).

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:420538 CAPLUS
DOCUMENT NUMBER: 129:146547
TITLE: **Immobilization** of saccharides and **peptides** on **96-well** microtiter **plates** coated with methyl vinyl ether-maleic anhydride copolymer
AUTHOR(S): Satōh, Ayano; Kojima, Kyoko; Koyama, Tamami; Ogawa, Haruko; Matsumoto, Isamu
CORPORATE SOURCE: Department of Chemistry, Faculty of Science, Ochanomizu University, Tokyo, 112-0012, Japan
SOURCE: Anal. Biochem. (1998), 260(1), 96-102
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have previously reported a method to **immobilize protein** ligands on microtiter plates coated with Me vinyl ether-maleic anhydride copolymer (MMAC). In this study, we improved the MMAC method to efficiently immobilize not only small ligands such as peptides and oligosaccharides, which could not be efficiently immobilized previously, but also heparin via its reducing end. Amino and hydrazino groups were introduced to MMAC-coated microtiter plate wells by coupling to acid anhydride groups of MMAC with 1,6-hexamethylenediamine and adipic acid dihydrazide, resp. The amino groups introduced were allowed to react with peptides by use of divalent cross-linkers. Hydrazino groups were allowed to react with formyl groups of saccharides by reductive amination. **Peptides** and oligosaccharides were **immobilized** in a dose-dependent manner by these methods. In the case of the angiotensin **peptide** thus **immobilized**, the detection limit by monoclonal antibodies was as low as 0.1-1 fmol peptide per well. Application of 20-200 nmol oligosaccharides to the well was sufficient to immobilize and subsequently detect lectins. Furthermore, heparin immobilized on the hydrazino-coated wells was successfully used for the binding assay of annexin IV. (c) 1998 Academic Press.

L18 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:353154 CAPLUS
DOCUMENT NUMBER: 129:25201
TITLE: An Assay Method for Evaluating Chemical Selectivity of Agonists for Insulin Signaling Pathways Based on Agonist-Induced Phosphorylation of a Target Peptide
AUTHOR(S): Ozawa, Takeaki; Sato, Moritoshi; Sugawara, Masao; Umezawa, Yoshio

CORPORATE SOURCE: Department of Chemistry School of Science, University of Tokyo, Tokyo, 113, Japan
SOURCE: Anal. Chem. (1998), 70(11), 2345-2352
CODEN: ANCHAM; ISSN: 0003-2700
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An optical method for evaluating the physiol. relevant agonist and antagonist selectivity of an insulin signaling pathway based on an insulin-dependent on/off switching of phosphorylation of a target peptide via insulin receptor is described. Insulin receptor serves as a binding for insulin and a given insulin receptor-binding peptide as a target for an insulin-receptor complex. Upon binding of insulin to its receptor, it undergoes autophosphorylation which enables the receptor to have a kinase activity and phosphorylate various substrates. The phosphorylated tyrosine in the substrate was measured with a monoclonal anti-phosphotyrosine antibody. As the target substrate for insulin receptor, a Y939 peptide consisting of 12 amino acid residues derived from insulin receptor substrate 1 (IRS-1) was used. The present assay method involves different sequential steps: (1) **immobilization** of a biotin-coupled Y939 **peptide** on an avidin coated **96-well plate** via biotin-avidin complexation; (2) insulin-dependent phosphorylation of the Y939 peptide by insulin receptor; (3) enzymic reaction and absorptiometric assay of the phosphorylated Y939 peptide using the anti-phosphotyrosine antibody labeled with horseradish peroxidase. An insulin-dependent absorbance was obsd. for insulin concns. from 1.0 .times. 10-10 to 1.0 .times. 10-7 M, and it leveled off. The obsd. absorbance was explained to be due to an increase in the phosphorylated Y939 peptide caused by insulin and its receptor complexation. No signal was, however, induced by both vanadyl and vanadate ions at concns. up to 1.0 .times. 10-4 M; these results and previous intact cell level data taken together led to the conclusion that these ions did not induce phosphorylation of the Y939 peptide. Upon addn. of tyrphostin, a specific inhibitor for insulin receptor kinase activity, phosphorylation of the Y939 peptide in the presence of 1.0 .mu.M insulin was competitively inhibited over 1.0 .times. 10-4 M tyrphostin. The present system thus exhibited "physiol. more relevant" agonist and antagonist selectivity, the principle of which is based in part on the insulin signal transduction rather than simply relying on the binding assay. The potential use of the present method for evaluating the selectivity of a wide range of agonists and antagonists toward the insulin signaling pathways is discussed.

L18 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:482773 CAPLUS
DOCUMENT NUMBER: 127:201658
TITLE: Active sites of salivary proline-rich protein for binding to Porphyromonas gingivalis fimbriae
AUTHOR(S): Kataoka, Kosuke; Amano, Atsuo; Kuboniwa, Masae; Horie, Hiroshi; Nagata, Hideki; Shizukuishi, Satoshi
CORPORATE SOURCE: Department of Preventive Dentistry, Osaka University Faculty of Dentistry, Suita, 565, Japan
SOURCE: Infect. Immun. (1997), 65(8), 3159-3164
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Porphyromonas gingivalis fimbriae specifically bind salivary acidic proline-rich protein 1 (PRP1) through protein-protein interactions. The binding domains of fimbrillin (a subunit of fimbriae) for PRP1 were analyzed previously (A. Amano, A. Sharma, J.-Y. Lee, H. T. Sojar, P. A. Raj, and R. J. Genco, Infect. Immun. 64:1631-1637, 1996). In this study, we investigated the sites of binding of the PRP1 mols. to the fimbriae. PRP1 (amino acid residues 1 to 150) was proteolyzed to three fragments (residues 1 to 74 [fragment 1-74], 75 to 129, and 130 to 150). 125I-labeled fimbriae clearly bound fragments 75-129 and 130-150, immobilized on a polyvinylidene difluoride membrane; both fragments also inhibited whole-cell binding to PRP1-coated hydroxyapatite (HAP) beads by 50 and 83%, resp. However, the N-terminal fragment failed to show any effect. Analogous peptides corresponding to residues 75 to 89, 90 to 106, 107 to 120, 121 to 129, and 130 to 150 of PRP1 were synthesized. The fimbriae significantly bound **peptide** 130-150, **immobilized** on **96-well plates,** and the peptide also inhibited binding of 125I-labeled fimbriae to PRP1-coated HAP beads by almost 100%. Peptides 75-89, 90-106, and 121-129, immobilized on plates, showed considerable ability to bind fimbriae. For further anal. of active sites in residues 130 to 150, synthetic peptides corresponding to residues 130 to 137, 138 to 145, and 146 to 150 were prepd. Peptide 138-145 (GRPQGPPQ) inhibited fimbrial binding to PRP1-coated HAP beads by 97%. This amino acid sequence was shared in the alignment of residues 75 to 89, 90 to 106, and 107 to 120. Six synthetic peptides were prepd. by serial deletions of individual residues from the N and C termini of peptide GRPQGPPQ. Peptide PQGPPQ was as inhibitory as peptide GRPQGPPQ. Further deletions of the dipeptide Pro-Gln from the N and C termini of peptide PQGPPQ resulted in significant loss of the inhibitory effect. These results strongly suggest that PQGPPQ is the minimal active segment for binding to P. gingivalis fimbriae and that the moiety of the Pro-Gln dipeptide plays a crit. role in expressing binding ability.

L18 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:193661 CAPLUS
DOCUMENT NUMBER: 124:229841
TITLE: Ligation of CD69 induces apoptosis and cell death in human eosinophils cultured with granulocyte-macrophage colony-stimulating factor
AUTHOR(S): Walsh, Garry M.; Williamson, Mairi L.; Symon, Fiona A.; Willars, Gary B.; Wardlaw, Andrew J.
CORPORATE SOURCE: Sch. Med., Leicester Univ., Leicester, UK
SOURCE: Blood (1996), 87(7), 2815-21
CODEN: BLOOAW; ISSN: 0006-4971
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Peripheral blood (PB) eosinophils rapidly undergo apoptosis and cell death in vitro unless cultured in the presence of cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) in which their survival is prolonged for up to 10 days. CD69 is a type II membrane antigen expressed by cytokine-activated, but not freshly isolated, PB human eosinophils. We have examd. the effect of ligation of CD69 by specific monoclonal antibody (MoAb) on the

viability of human eosinophils cultured with recombinant human (rh)GM-CSF. Eosinophils were purified by immunomagnetic selection and cultured with GM-CSF (10-10 mol/L). Eighteen hours after the start of culture, a panel of CD69 MoAb or controls (anti-CR3 or isotype-matched control MoAb) were added. Viability was assessed by trypan blue exclusion and apoptosis by morphol. assessment, DNA laddering, and flow cytometric anal. of eosinophil red autofluorescence. Up to 50% of the eosinophils had undergone apoptosis 48 h after addn. of anti-CD69 MoAb compared with less than 10% apoptosis for CR3 or the isotype matched control. The majority of apoptotic eosinophils excluded trypan blue at 48 h post CD69 ligation. More apoptotic eosinophils were obsd. at later time-points and this was assocd. with loss of viability. At 120 h post-addn. of the anti-CD69 MoAb MLR3, 24% eosinophils were viable compared with 84% for the CR3 control. A F(ab)2 fragment of CD69 MoAb P8, also induced apoptosis in GM-CSF cultured eosinophils. A more rapid induction of eosinophil apoptosis was obtained with CD69 MoAb **immobilized** via their Fc portions on **protein** -A coated plastic **96 well plates**. Ligation of CD69 or CR3 resulted in the release of comparable quantities of eosinophil peroxidase at 48 h post-ligation. These levels of EPO were consistent with the viability of these cells at 48 h as assessed by exclusion of trypan blue. Finally, a neutralizing MoAb to TGF.beta.1 had no effect on CD69-dependent apoptosis induction nor were there detectable quantities of TGF.beta.1 in supernatants from GM-CSF-cultured eosinophils ligated with CD69 or control MoAb. These results suggest that eosinophils cultured with GM-CSF can be induced to undergo apoptosis as a result of cell signalling mediated by perturbation of CD69. This may represent an important physiol. mechanism for eosinophil removal in vivo.

L18 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:654037 CAPLUS
DOCUMENT NUMBER: 121:254037
TITLE: Detection of goats' milk in ewes' milk by an indirect ELISA
AUTHOR(S): Garcia, T.; Martin, R.; Rodriguez, E.; Morales, P.; Gonzalez, I.; Sanz, B.; Hernandez, P. E.
CORPORATE SOURCE: Facultad de Veterinaria, Universidad Complutense, Madrid, 28040, Spain
SOURCE: Food Agric. Immunol. (1994), 6(1), 113-18
CODEN: FAIMEZ; ISSN: 0954-0105
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An indirect ELISA has been developed successfully for the detection of defined amts. of goats' milk (1-100%) in ewes' milk. The assay uses polyclonal antibodies against goats' whey proteins (GWP) raised in rabbits. The anti-GWP antibodies were recovered from the crude antiserum by immunoadsorption and elution from a column contg. immobilized GWP. The anti-GWP antibodies were biotinylated and rendered goats' milk-specific by mixing them with lyophilized cows' and ewes' whey proteins. ExtrAvidin-peroxidase was used to detect the specific anti-GWP antibodies bound to goats' milk **proteins immobilized** on **96-well plates**. Subsequent enzymic conversion of substrate resulted in discernible differences in optical d. between mixts. of ewes' milk contg. variable amts. of goats' milk.

L18 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:293570 CAPLUS
DOCUMENT NUMBER: 120:293570
TITLE: Sorbent families of peptides for affinity chromatography supports and labeled reagents
INVENTOR(S): Kauvar, Lawrence M.
PATENT ASSIGNEE(S): Terrapin Technologies, Inc., USA
SOURCE: PCT Int. Appl., 47 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9402225	A1	19940203	WO 1993-US6578	19930713
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 652798	A1	19950517	EP 1993-917148	19930713
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 07509400	T2	19951019	JP 1993-504528	19930713
AU 675446	B2	19970206	AU 1993-46758	19930713
US 5599901	A	19970204	US 1994-248538	19940524
US 5801225	A	19980901	US 1996-690605	19960731

PRIORITY APPLN. INFO.: US 1992-920335 19920727
WO 1993-US6578 19930713
US 1994-248538 19940524

AB Compds. useful as affinity chromatog. supports and as labeled reagents are disclosed. The compds. are peptides which can be constituted in families of pos. charged, neg. charged or uncharged small peptides or the amidated forms thereof with varying characteristics as to charge, charge distribution, hydrophobicity, cyclization, and helical conformation propensity. Paralog sorbents were synthesized as individual paralog peptides and coupled to N-hydroxysuccinimide-activated agarose (Affi-Gel 10). A slurry of each sorbent was placed in replicate wells of a membrane-bottomed flow-through **96-well** test **plate**, creating miniature columns. The sorbents were used in the sequential fractionation of a complex protein mixt. from a whole cell acetone lysate of yeast.

L18 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:631705 CAPLUS
DOCUMENT NUMBER: 117:231705
TITLE: A method for rapid screening of recombinant proteins for recognition by T lymphocytes
AUTHOR(S): Hickling, Julian K.; Jones, K. Rebecca; Yuan, Bin; Rothbard, Jonathan B.; Bulow, Roland
CORPORATE SOURCE: ImmuLogic Pharm. Corp., Palo Alto, CA, 94304, USA
SOURCE: Eur. J. Immunol. (1992), 22(8), 1983-7
CODEN: EJIMAF; ISSN: 0014-2980
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A simple, cost-effective method is described that allows rapid screening of recombinant protein sequences for their ability to stimulate T cells. Individual microcultures of E. coli each expressing a gene product or peptide sequence fused to protein A are grown in **96-well plates**. Following lysis of the bacteria, the fusion **peptide** is readily captured with **immobilized** Ig in tissue culture wells. No further purifn. is required. T lymphocytes plus appropriate antigen-presenting cells are added directly to the wells and assayed for proliferation. The DNA in bacteria from wells stimulating T cell proliferation is then sequenced. The technique allows rapid mapping of T cell epitopes by facilitating screening of truncation mutants without extensive purifn. Described here is a further application of the technique to study monosubstituted analogs of a known T cell epitope.

L18 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:570648 CAPLUS
DOCUMENT NUMBER: 111:170648
TITLE: Method and kit for the competitive immunoassay of apolipoproteins using **immobilized** antibody and antigenic hybrid label **protein**
INVENTOR(S): Baralle, Francisco Ernesto; Sidoli, Alessandro
PATENT ASSIGNEE(S): Istituto Sieroterapico Milanese S. Belfanti, Italy
SOURCE: Eur. Pat. Appl., 21 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 301667	A1	19890201	EP 1988-201622	19880727
R: AT, BE, CH, DE, ES, FR, GB, IT, LI, LU, NL, SE				
WO 8901164	A1	19890209	WO 1988-GB616	19880728
W: JP, US				
GB 2208317	A1	19890322	GB 1988-18031	19880728
JP 02500164	T2	19900125	JP 1988-506237	19880728
PRIORITY APPLN. INFO.:			GB 1987-17791	19870728
			WO 1988-GB616	19880728

AB Apolipoprotein is detected or estd. in a sample by (a) contacting the sample with a solid support having immobilized antibody to apolipoprotein and with a fused protein comprising an antigenic part of the apolipoprotein and a label protein; and (b) observing or measuring the label protein either bound or not bound to the support. A test hit comprises the **immobilized** antibody and the hybrid **protein**. The assay is called RIECA (Recombinant immuno Enzymic Competition Assay). Apolipoproteins A-I and B was detd. in whole blood, serum, and plasma using specific monoclonal antibodies immobilized in **96-well plates** and .beta.-galactosidase fusion proteins [prepd. by expression of Escherichia coli plasmid pISMAI (coding for the enzyme and for apo-A-I) or plasmid pISMBI (coding for the enzyme and coding sequences of apolipoprotein B-1).

[redacted] BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH,
[redacted] ENTERED AT 11:59:57 ON 24 APR 2002)
L19 48 S L17
[redacted] 46 S L19 NOT L12
[redacted] (22 DUPLICATES REMOVED)

L21 ANSWER 1 OF 24 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-122551 [16] WPIDS
DOC. NO. CPI: C2002-037587
TITLE: Isolated nucleic acids comprising specific polynucleotide sequences and/or consisting polynucleotide which encode novel human B-7 like polypeptides for diagnosis and treatment of e.g. inflammation.
DERWENT CLASS: B04 D16
INVENTOR(S): KUZUHARA, H; MATSUOKA, K; NATORI, Y; NISHIKAWA, K; SUZUKI, Y; TERUNUMA, T
PATENT ASSIGNEE(S): (NISC-N) JAPAN SCI & TECHNOLOGY CORP
COUNTRY COUNT: 22
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002002588	A1	20020110	(200216)*	JA	493

RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: JP KR US

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002002588	A1	WO 2001-JP5717	20010702

PRIORITY APPLN. INFO: JP 2001-75081 20010315; JP 2000-200139 20000630
AN 2002-122551 [16] WPIDS
AB WO 200202588 A UPAB: 20020308
NOVELTY - An isolated nucleic acid molecule comprising: (a) polynucleotide of SEQ ID NO:X or polynucleotide encoded by cDNA; (b) polynucleotide encoding biologically active polypeptide fragment of SEQ ID NO:Y or polypeptide fragment encoded by cDNA; (c) polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or polypeptide epitope encoded by cDNA; or (d) polynucleotide capable of hybridizing.

DETAILED DESCRIPTION - An isolated nucleic acid molecule (I) comprising:

(a) polynucleotide of SEQ ID NO:X or encoded by cDNA included in American Type Culture Collection (ATCC) Deposit No:Z;

(b) polynucleotide encoding biologically active polypeptide fragment of SEQ ID NO:Y or biologically active polypeptide fragment encoded by cDNA sequence included in ATCC Deposit No:Z;

(c) polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or polypeptide epitope encoded by cDNA sequence included in ATCC Deposit No:Z; or

(d) polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(c), but does not hybridize under stringent conditions to nucleic acid

molecule having a nucleotide sequence of only A residues or T residues.

INDEPENDENT CLAIMS are also included for:

(1) an isolated polypeptide (II) comprising an amino acid sequence at least 95% identical to a sequence consisting of :

(i) polypeptide of SEQ ID NO:Y or the polypeptide encoded by cDNA;

(ii) polypeptide fragment of SEQ ID NO:Y or the polypeptide encoded by cDNA;

(iii) polypeptide epitope of SEQ ID NO:Y or the polypeptide encoded by cDNA; and

(iv) a variant of SEQ ID NO:Y;

(2) a vector (V1) comprising (I);

(3) a host cell comprising the vector (V1);

(4) a recombinant host cell (RC1) comprising (I) operably limited to heterologous regulating element which controls gene expression;

(5) a method for producing a polypeptide comprising expressing encoded polypeptide from the recombinant host cell as in (4) above, and recovering the polypeptide;

(6) an isolated antibody that binds specifically to the isolated polypeptide (II);

(7) a recombinant host cell (RC2) that expresses the isolated polypeptide (II);

(8) a method of making an isolated polypeptide comprising:

(i) culturing RC2 under conditions such that the polypeptide is expressed; and

(ii) recovering the polypeptide;

(9) a method of diagnosing a pathological condition or a susceptibility to a pathological condition comprising:

(i) determining the presence or amount of expression of (II) in a biological sample; and

(ii) diagnosing a pathological condition or susceptibility to a pathological condition based on the presence or amount of expression of (II);

(10) a method for identifying a binding partner to (II) comprising:

(i) contacting (II) with a binding partner; and

(ii) determining whether the binding partner effects an activity of (II);

(11) a method of screening for molecules which modify activities of the (II) comprising:

(i) contacting (II) with a compound suspected of having agonist or antagonist activity; and

(ii) assaying for activity of (II).

X may be any of the polynucleotide sequences disclosed in sequence listing and Y may be any of the polypeptide sequences disclosed in sequence listing.

ACTIVITY - Antiinflammatory; Immunomodulatory.

MECHANISM OF ACTION - Modulation and costimulation of interactions between B-7 ligands and their receptors;

Polypeptide costimulation of T cells.

A costimulation assay on peripheral blood lymphocytes (PBL) was performed in the presence of immobilized antibodies CD3 and CD28. The use antibodies specific for the invariant regions of CD3 mimic the induction of T cell activation that would occur through the stimulation of the T cell receptor by an antigen. The addition of costimulatory signal such as an antibody to CD28, which mimics the

action of costimulatory molecule B7-1 results in enhancement of T cell responses, including cell survival and production of IL-2. The assay was performed in a 96 well plate and using 2 multiply 104cells/well in a final volume of 200 micro l. A supernatant expressing B7-like polypeptides of interest was tested at a final 30% final dilution. Control supernatants were used to at the same final dilution and expressed the following proteins: vector only (negative control), IL-2, IFN-gamma, TNF-alpha, IL-10 and TR2. The assay allowed detection of both positive and negative effects caused by addition of supernatants expressing the proteins of interest on T cell proliferation.

USE - (I) and (II) are used for preventing, treating or ameliorating a medical condition in a mammalian subject (claimed). The polynucleotides and polypeptides are administered to subjects having a disorder related to B-7 Like polypeptides, such as inappropriate or excessive inflammation which can lead to tissue damage or even death, where the inflammation is brought about by the activation of certain cells in the body e.g. T cells and may involve disorders related to immune system. The polynucleotides can also serve as probes or primers in chromosome identification, chromosome mapping and linkage analysis.

ADVANTAGE - The polynucleotides and polypeptides provide more effective and less toxic alternatives to steroidal and nonsteroidal drugs, for modulating inflammatory response.

Dwg.0/0

L21 ANSWER 2 OF 24 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-556579 [62] WPIDS
CROSS REFERENCE: 1995-090609 [12]; 1998-178542 [16]; 1999-457113 [38]
DOC. NO. CPI: C2001-165460
TITLE: Biotinylation of **proteins** and **immobilizing** them on substrates for us in diagnosis and research.
DERWENT CLASS: B04 D16
INVENTOR(S): SCHATZ, P J
PATENT ASSIGNEE(S): (AFFY-N) AFFYMAX TECHNOLOGIES NV
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 6265552	B1	20010724	(200162)*		36

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 6265552	B1	CIP of	US 1993-99991	19930730
		Cont of	US 1995-383753	19950203
		Cont of	US 1997-959512	19971028
		Cont of	US 1999-267900	19990311
			US 2000-512983	20000224

FILING DETAILS:

PATENT NO	KIND	PATENT NO

US 6265552 B1 Cont of US 5723584
Cont of US 5932433

PRIORITY APPLN. INFO: US 1995-383753 19950203; US 1993-99991 19930730; US 1997-959512 19971028; US 1999-267900 19990311; US 2000-512983 20000224

AN 2001-556579 [62] WPIDS
CR 1995-090609 [12]; 1998-178542 [16]; 1999-457113 [38]
AB US 6265552 B UPAB: 20011026
NOVELTY - A method for **immobilizing** and biotinylating a **protein** on a substrate, is new.

DETAILED DESCRIPTION - A method for **immobilization** of a **protein**, comprising:

(1) providing avidin and/or streptavidin on a surface of a substrate;

(2) constructing a recombinant DNA expression vector that encodes a fusion protein, which comprises the protein and a biotinylation peptide comprising the amino acid sequence (A1) (the biotinylation peptide is capable of being biotinylated by a biotin ligase at the lysine residue adjacent to Xaa6 and is 13-150 amino acids in length);

(3) transforming a recombinant host cell with the vector;

(4) culturing the host cell in the presence of biotin or a biotin analog and under conditions so that the fusion protein and a biotinylation enzyme are expressed, resulting in biotinylation of the fusion protein; and

(5) contacting the biotinylated fusion protein with a pre-defined region of the surface so that the biotinylated fusion **proteins** is **immobilized** on the substrate.

Leu-Xaa1-Xaa2-Ile-Xaa3-Xaa4-Xaa5-Xaa6-Lys-Xaa7-Xaa8-Xaa9-Xaa10 (A1)

Xaa1 = any amino acid;
Xaa2 = any amino acid other than Leu, Val, Ile, Trp, Phe or Tyr;
Xaa3 = Phe or Leu;
Xaa4 = Glu or Asp;
Xaa5 = Ala, Gly, Ser and/or Thr;
Xaa6 = Gln or Met;
Xaa7 = Ile, Met or Val;
Xaa8 = Glu, Leu, Val, Tyr or Ile;
Xaa9 = Trp, Tyr, Val, Phe, Leu or Ile; and
Xaa10 = any amino acid other than Asp, Glu.

USE - The biotinylated **proteins immobilized** on the substrate may be used in standard laboratory techniques research and diagnosis, e.g. for rapid purification, **immobilization**, labeling and detecting the **proteins**.

ADVANTAGE - The method is a simple and effective way of biotinylating proteins.

Dwg.0/0

L21 ANSWER 3 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 1
ACCESSION NUMBER: 2001:546559 BIOSIS
DOCUMENT NUMBER: PREV200100546559
TITLE: Simple dip strip ELISA for airborne estrogenic steroids.
AUTHOR(S): Armstrong, Stewart; Miao, Z.-F.; Rowell, Frederick J.

(1); Ali, Zulfiqur
CORPORATE SOURCE: (1) North East Biotechnology Centre, School of Sciences, Institute of Pharmacy and Chemistry, University of Sunderland, Sunderland, SR1 3SD: frederick.rowell@sunderland.ac.uk UK
SOURCE: Analytica Chimica Acta, (12 October, 2001) Vol. 444, No. 1, pp. 79-86. print.
ISSN: 0003-2670.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB An ELISA for the potent estrogenic steroids, ethinyl estradiol (ETED), 17-beta-estradiol (ED) and estrone (ES) has been developed and used to determine the recovery of ED and ETED following spiking from seven filters commonly used in samplers for ascertaining personal exposure of workers to airborne biochemicals. Best results were obtained with cellulose nitrate (CN) and polytetrafluoroethylene (PTFE) filters. The assay reagents have also been used to develop a simple dip strip assay that can be used to determine the presence of steroids on the filters. Steroids captured from the air on the surface of filters within a conventional personal exposure sampler are extracted with specific antiserum. A spot of hapten-**protein** conjugate is **immobilised** on a small square of CN filter attached to a plastic strip. This is immersed in the sampler solution where unbound antibodies bind to the hapten on the spot. The strips are then transferred to a **96 well** filter **plate** located within a filter manifold. Following washing, strips are incubated with alkaline phosphatase-labelled second antibody and the spots were developed by addition of substrate. The intensity of the developed blue spot is inversely proportional to the amount of steroid originally captured on the filter. A batch of over 50 samplers can be screened within 1 h. Spots on strips from filters on which 10 ng of ED, ES or ETED are present can be visually discerned from strips from filters where no steroid is present.

L21 ANSWER 4 OF 24 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001666887 IN-PROCESS
DOCUMENT NUMBER: 21569096 PubMed ID: 11712696
TITLE: Immunofluorscence--still the 'gold standard' in ANA testing?.
AUTHOR: Kumagai S; Hayashi N
CORPORATE SOURCE: Department of Clinical and Laboratory Medicine, Kobe University School of Medicine, Hyogo, Japan.. kumagais@kobe-u.ac.jp
SOURCE: SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION. SUPPLEMENT, (2001) (235) 77-83.
Journal code: 2984789R. ISSN: 0085-591X.
PUB. COUNTRY: Norway
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20011120
Last Updated on STN: 20020123
AB A usefulness of enzyme immunoassay (EIA)-based antinuclear antibodies (ANA) tests was evaluated in comparison with the immunofluorescence ANA assay (IF-ANA). COBAS-ANA and MBL-ANA were used, in the former a mixture of antigens extracted from HEp-2 cells

and multiple recombinant antigens was immobilized on beads as the antigen, and in the latter 9 kinds of purified or recombinant **proteins** are **immobilized** on **96-well plates**. We first compared an ability to differentiate 258 connective tissue disease (CTD) patients (except rheumatoid arthritis) from 257 healthy subjects between COBAS-ANA and IF-ANA. The sensitivity and specificity of COBAS-ANA were 84% and 94%, respectively, while those of IF-ANA at a cutoff dilution of 1:160 were 81% and 87%. The receiver operating characteristic (ROC) analysis showed a significant superiority of COBAS-ANA to IF-ANA. Moreover, when the cutoff index was set at 0.6, the COBAS-ANA could detect the 8 disease-specific ANAs as well as IF-ANA at a cutoff dilution of 1:40. A possible availability of MBL-ANA in a periodic health examination in certain towns was also demonstrated.Among the 1123 subjects, a total of 145 disease-specific ANAs were detected in 126 subjects. MBL-ANA could catch disease-specific ANAs with almost same efficacy of IF-ANA. Annual survey of the residents by MBL-ANA may lead to a detection of CTD patients. EIA-based ANA tests are very useful for both detecting disease-specific ANAs and screening CTD patients. We believe that EIA-ANA should be the 'gold standard' especially for screening a large number of samples, although there is some room for technical improvement.

L21 ANSWER 5 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 3

ACCESSION NUMBER: 2001:559782 BIOSIS
DOCUMENT NUMBER: PREV200100559782
TITLE: Immunofluorescence: Still the 'gold standard' in ANA testing.
AUTHOR(S): Kumagai, Shunichi (1); Hayashi, Nobuhide
CORPORATE SOURCE: (1) Dept Clin and Lab Medicine, Kobe Univ School of Medicine, 7-5-2 Kusunoki-cyo, Chuou-ku, Kobe, Hyogo, 650-0017: kumagais@kobe-u.ac.jp Japan
SOURCE: Scandinavian Journal of Clinical and Laboratory Investigation, (2001) Vol. 61, No. Supplement 235, pp. 77-83. print.
ISSN: 0036-5513.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A usefulness of enzyme immunoassay (EIA)-based antinuclear antibodies (ANA) tests was evaluated in comparison with the immunofluorescence ANA assay (IF-ANA). COBAS-ANA and MBL-ANA were used, in the former a mixture of antigens extracted from HEp-2 cells and multiple recombinant antigens was immobilized on beads as the antigen, and in the latter 9 kinds of purified or recombinant **proteins** are **immobilized** on **96-well plates**. We first compared an ability to differentiate 258 connective tissue disease (CTD) patients (except rheumatoid arthritis) from 257 healthy subjects between COBAS-ANA and IF-ANA. The sensitivity and specificity of COBAS-ANA were 84% and 94%, respectively, while those of IF-ANA at a cutoff dilution of 1:160 were 81% and 87%. The receiver operating characteristic (ROC) analysis showed a significant superiority of COBAS-ANA to IF-ANA. Moreover, when the cutoff index was set at 0.6, the COBAS-ANA could detect the 8 disease-specific ANAs as well as IF-ANA at a cutoff dilution of 1:40. A possible availability of MBL-ANA in a periodic health examination in certain towns was also demonstrated. Among the

1123 subjects, a total of 145 disease-specific ANAs were detected in 126 subjects. MBL-ANA could catch disease-specific ANAs with almost same efficacy of IF-ANA. Annual survey of the residents by MBL-ANA may lead to a detection of CTD patients. EIA-based ANA tests are very useful for both detecting disease-specific ANAs and screening CTD patients. We believe that EIA-ANA should be the 'gold standard especially for screening a large number of samples, although there is some room for technical improvement.

L21 ANSWER 6 OF 24 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000046671 EMBASE
TITLE: A high-throughput assay to identify compounds that can induce dimerization of the erythropoietin receptor.
AUTHOR: Biazzo D.E.; Motamedi H.; Mark D.F.; Qureshi S.A.
CORPORATE SOURCE: S.A. Qureshi, Merck Research Laboratories, MS RY80Y-310, P.O. Box 2000, Rahway, NJ 07065, United States
SOURCE: Analytical Biochemistry, (1 Feb 2000) 278/1 (39-45). Refs: 31
ISSN: 0003-2697 CODEN: ANBCA2
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Erythropoietin induces dimerization of the erythropoietin receptor on the surface of erythroid progenitor cells, promoting the differentiation of these cells into mature red blood cells. To facilitate screening of large chemical collections for identification of compounds that can dimerize erythropoietin receptor, we have developed a novel, high-throughput in vitro assay to detect compounds that can cause dimerization of the erythropoietin receptor in solution. To develop this assay, amino acid sequences corresponding to the extracellular domain of erythropoietin receptor were expressed in Escherichia coli as erythropoietin-binding protein (rEBP). A modified version of this protein (33P-rEBP) containing a protein kinase A substrate site incorporated into the rEBP was also expressed in E. coli and labeled in vitro using protein kinase A and [.gamma.-33P]ATP. An erythropoietin mimetic peptide (EMP1), that induces dimerization of rEBP in solution was used to demonstrate dimerization of 33P-rEBP and rEBP in a **96-well** microtiter **plate** format. EMP-1 induced dimerization of rEBP in this assay with an EC50 of .apprx.245 nM and had a maximal effect at 0.5-2 .mu.M and required the presence of rEBP immobilized on the **plate** capable of binding EMP-1. EMP- 1-induced dimerization of 33P-rEBP and rEBP was reversed by excess unlabeled rEBP and was not masked by complex mixtures such as whole cell fungal extracts. These data demonstrate the ability of 33P-rEBP to dimerize with rEBP in vitro in a format that is fully compatible with high-throughput screening.

L21 ANSWER 7 OF 24 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1999274073 MEDLINE
DOCUMENT NUMBER: 99274073 PubMed ID: 10344276
TITLE: Mapping and identification of interferon gamma-regulated HeLa cell **proteins** separated by **immobilized** pH gradient

two-dimensional gel electrophoresis.

AUTHOR: Shaw A C; Rossel Larsen M; Roepstorff P; Justesen J; Christiansen G; Birkelund S

CORPORATE SOURCE: Department of Medical Microbiology and Immunology, University of Aarhus, Denmark.. shaw@medmicro.aau.dk

SOURCE: ELECTROPHORESIS, (1999 Apr-May) 20 (4-5) 984-93.
Journal code: ELE; 8204476. ISSN: 0173-0835.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199907

ENTRY DATE: Entered STN: 19990806
Last Updated on STN: 20000303
Entered Medline: 19990729

AB Interferon gamma (IFN-gamma) is a potent immunomodulatory lymphokine, secreted by activated T-lymphocytes and NK-cells during the cellular immune response. Actions of IFN-gamma are mediated through binding to the IFN-gamma-receptor, present on most cells, and the subsequent activation of a great magnitude of IFN-gamma responsive genes has been reported previously. Our goal is to identify and map IFN-gamma-regulated HeLa cell proteins to the two-dimensional polyacrylamide gel electrophoresis with the immobilized pH gradient (IPG) two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) system. A semiconfluent layer of HeLa cells was grown on tissue culture **plates**, and changes in protein expression due to 100 U/mL IFN-gamma were investigated at different periods after treatment, using pulse labeling with [35S]methionine/cysteine in combination with 2-D PAGE (IPG). The identity of eight protein spots was elucidated by **matrix** -assisted **laser** desorption/**ionization**-mass spectrometry (**MALDI**-MS), and several variants of the IFN-gamma-inducible tryptophanyl-tRNA synthetase (hWRS) were detected by immunoblotting.

L21 ANSWER 8 OF 24 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 1999153058 EMBASE

TITLE: A rapid and sensitive quantitative kinase activity assay using a convenient 96-well format.

AUTHOR: Asthagiri A.R.; Horwitz A.F.; Lauffenburger D.A.

CORPORATE SOURCE: D.A. Lauffenburger, Department of Chemical Engineering, Div. of Bioengineering/Envtl. Health, Massachusetts Inst. of Technology, Cambridge, MA 02139, United States. lauffen@mit.edu

SOURCE: Analytical Biochemistry, (15 Apr 1999) 269/2 (342-347).
Refs: 9
ISSN: 0003-2697 CODEN: ANBCA2

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Activation of protein kinases in response to growth factor and extracellular matrix stimulation has been implicated in regulating a number of cell functions including differentiation, gene expression, migration, and proliferation. An improved quantitative assay for measuring protein kinase activity is crucial to the detailed study

of this important category of signaling proteins and their role in regulating cell behavior. We describe a modified in vitro kinase activity assay that is both sensitive and quantitative. It offers several advantages when compared to the traditional immunoprecipitation/kinase assay: (i) high sensitivity that reduces the required amount of cell lysate by an order of magnitude, (ii) an immunoseparation technique utilizing antibody immobilization onto the surface of microtiter wells that replaces the cumbersome immunoprecipitation method, (iii) a **96-well plate** configuration that eases handling of multiple samples and increases throughput of the assay, and (iv) the use of **96-well** filter **plates** that greatly reduces radioactive liquid waste generation. While we implement this technique in a case study for measuring the activity of extracellular signal-regulated kinase 2 (ERK2), this assay can be extended to studying other protein kinases by using an appropriate antibody and in vitro substrate for the kinase of interest.

L21 ANSWER 9 OF 24 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999105424 EMBASE
TITLE: Increasing the sensitivity of Listeria monocytogenes assays: Evaluation using ELISA and amperometic detection.
AUTHOR: Crowley E.L.; O'Sullivan C.K.; Guilbault G.G.
CORPORATE SOURCE: G.G. Guilbault, Laboratory of Sensor Development, National University of Ireland Cork, Cork, Ireland
SOURCE: Analyst, (1999) 124/3 (295-299).
Refs: 15
ISSN: 0003-2654 CODEN: ANALAO
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
049 Forensic Science Abstracts
052 Toxicology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB An immunosensor for the detection of Listeria monocytogenes was developed. ELISA and amperometric studies were run in parallel to develop a more sensitive and rapid assay for the bacterium. Conditions for the immunosensor were primarily characterised using ELISA. A direct sandwich assay was employed and the affinities of two polyclonal (goat and rabbit) and one monoclonal (mouse) anti-L. monocytogenes antibodies were compared using this format. Owing to low sensitivity being obtained with all antibodies, biotin-avidin amplification and an indirect sandwich assay were employed. The system was then transferred to screen-printed electrodes (SPEs), the primary antibody being immobilised by cross-linking with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, and the mode of detection being amperometric. Various parameters (limit of detection, working range, incubation time, cross-reactivity) of the systems were characterised. The effect of direct incubation in milk is also discussed. The final immunosensor had a working range of 1 x 106-1 x 103 cells ml-1 and a detection limit of 9 x 102 cells ml-1. The assay took about 3.5 h to complete.

L21 ANSWER 10 OF 24 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 1998288002 MEDLINE
DOCUMENT NUMBER: 98288002 PubMed ID: 9624906
TITLE: An assay method for evaluating chemical selectivity of agonists for insulin signaling pathways based on agonist-induced phosphorylation of a target peptide.
AUTHOR: Ozawa T; Sato M; Sugawara M; Umezawa Y
CORPORATE SOURCE: Department of Chemistry, School of Science, University of Tokyo, Japan.
SOURCE: ANALYTICAL CHEMISTRY, (1998 Jun 1) 70 (11) 2345-52.
Journal code: 4NR; 0370536. ISSN: 0003-2700.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980910
Last Updated on STN: 20000303
Entered Medline: 19980902

AB An optical method for evaluating the physiologically relevant agonist and antagonist selectivity of an insulin signaling pathway based on an insulin-dependent on/off switching of phosphorylation of a target peptide via insulin receptor is described. Insulin receptor serves as a binding for insulin and a given insulin receptor-binding peptide as a target for an insulin-receptor complex. Upon binding of insulin to its receptor, the insulin receptor undergoes autophosphorylation which enables the receptor to have a kinase activity and phosphorylate various substrates. The phosphorylated tyrosine in the substrate was measured with a monoclonal anti-phosphotyrosine antibody. As the target substrate for insulin receptor, a Y939 peptide consisting of 12 amino acid residues derived from insulin receptor substrate 1 (IRS-1) was used. The present assay method involves different sequential steps: (1) **immobilization** of a biotin-coupled Y939 **peptide** on an avidin coated **96-well plate** via biotin-avidin complexation; (2) insulin-dependent phosphorylation of the Y939 peptide by the insulin receptor; (3) enzymatic reaction and absorptiometric assay of the phosphorylated Y939 peptide using the anti-phosphotyrosine antibody labeled with horseradish peroxidase. An insulin-dependent absorbance was observed for insulin concentrations from 1.0 x 10(-10) to 1.0 x 10(-7) M, and it leveled off. The observed absorbance was explained to be due to an increase in the phosphorylated Y939 peptide caused by insulin and its receptor complexation. No signal was, however, induced by both vanadyl and vanadate ions at concentrations up to 1.0 x 10(-4) M; these results and previous intact cell level data taken together led to the conclusion that these ions did not induce phosphorylation of the Y939 peptide. Upon addition of tyrphostin 25, a specific inhibitor for insulin receptor kinase activity, phosphorylation of the Y939 peptide in the presence of 1.0 microM insulin was competitively inhibited over 1.0 x 10(-4) M tyrphostin 25. The present system thus exhibited "physiologically more relevant" agonist and antagonist selectivity, the principle of which is based in part on the insulin signal transduction rather than simply relying on the binding assay. The potential use of the present method for evaluating the selectivity of a wide range of agonists and antagonists toward the insulin signaling pathways is discussed.

L21 ANSWER 11 OF 24 EMBASE

ACCESSION NUMBER: 1998298112 EMBASE
TITLE: Mass-sensing, multianalyte microarray immunoassay with imaging detection.
AUTHOR: Silzel J.W.; Cercek B.; Dodson C.; Tsay T.; Obremski R.J.
CORPORATE SOURCE: J.W. Silzel, Beckman Coulter, Inc., 200 South Kraemer Boulevard, Brea, CA 92822-8000, United States. jsilzel@beckman.com
SOURCE: Clinical Chemistry, (1998) 44/9 (2036-2043).
Refs: 10
ISSN: 0009-9147 CODEN: CLCHAU
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
036 Health Policy, Economics and Management
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Miniaturization of ligand binding assays may reduce costs by decreasing reagent consumption, but it is less apparent that miniaturized assays can simultaneously exceed the sensitivity of macroscopic techniques by analyte 'harvesting' to exploit the total analyte mass available in a sample. Capture reagents (avidin or antibodies) immobilized in 200-.mu.m diameter zones are shown to substantially deplete analyte from a liquid sample during a 1-3-h incubation, and the assays that result sense the total analyte mass in a sample rather than its concentration. Detection of as few as 105 molecules of analyte per zone is possible by fluorescence imaging in situ on the solid phase using a near-infrared dye label. Single and multianalyte mass-sensing sandwich array assays of the IgG subclasses show the sensitivity and specificity of ELISA methods but use less than 1/100 the capture antibody required by the **96-well plate** format.

L21 ANSWER 12 OF 24 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1998312478 MEDLINE
DOCUMENT NUMBER: 98312478 PubMed ID: 9648659
TITLE: **Immobilization** of saccharides and **peptides** on **96-well** microtiter **plates** coated with methyl vinyl ether-maleic anhydride copolymer.
AUTHOR: Satoh A; Kojima K; Koyama T; Ogawa H; Matsumoto I
CORPORATE SOURCE: Department of Chemistry, Faculty of Science, Ochanomizu University, Tokyo, Japan.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1998 Jun 15) 260 (1) 96-102.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980921
AB We have previously reported a method to **immobilize**

protein ligands on microtiter plates coated with methyl vinyl ether-maleic anhydride copolymer (MMAC) [Isosaki, K., et al. (1992) J. Chromatogr. 597, 123-128]. In this study, we improved the MMAC method to efficiently immobilize not only small ligands such as peptides and oligosaccharides, which could not be efficiently immobilized previously, but also heparin via its reducing end. Amino and hydrazino groups were introduced to MMAC-coated microtiter plate wells by coupling to acid anhydride groups of MMAC with 1,6-hexamethylenediamine and adipic acid dihydrazide, respectively. The amino groups introduced were allowed to react with peptides by use of divalent cross-linkers. Hydrazino groups were allowed to react with formyl groups of saccharides by reductive amination. **Peptides** and oligosaccharides were **immobilized** in a dose-dependent manner by these methods. In the case of the angiotensin **peptide** thus **immobilized**, the detection limit by monoclonal antibodies was as low as 0.1-1 fmol peptide per well. Application of 20-200 nmol oligosaccharides to the well was sufficient to immobilize and subsequently detect lectins. Furthermore, heparin immobilized on the hydrazinocoated wells was successfully used for the binding assay of annexin IV.

L21 ANSWER 13 OF 24 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 97378088 MEDLINE
DOCUMENT NUMBER: 97378088 PubMed ID: 9234769
TITLE: Active sites of salivary proline-rich protein for binding to Porphyromonas gingivalis fimbriae.
AUTHOR: Kataoka K; Amano A; Kuboniwa M; Horie H; Nagata H; Shizukuishi S
CORPORATE SOURCE: Department of Preventive Dentistry, Osaka University Faculty of Dentistry, Suita, Japan.
SOURCE: INFECTION AND IMMUNITY, (1997 Aug) 65 (8) 3159-64. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970825
Last Updated on STN: 19970825
Entered Medline: 19970814

AB Porphyromonas gingivalis fimbriae specifically bind salivary acidic proline-rich protein 1 (PRP1) through protein-protein interactions. The binding domains of fimbrillin (a subunit of fimbriae) for PRP1 were analyzed previously (A. Amano, A. Sharma, J.-Y. Lee, H. T. Sojar, P. A. Raj, and R. J. Genco, Infect. Immun. 64:1631-1637, 1996). In this study, we investigated the sites of binding of the PRP1 molecules to the fimbriae. PRP1 (amino acid residues 1 to 150) was proteolysed to three fragments (residues 1 to 74 [fragment 1-74], 75 to 129, and 130 to 150). 125I-labeled fimbriae clearly bound fragments 75-129 and 130-150, immobilized on a polyvinylidene difluoride membrane; both fragments also inhibited whole-cell binding to PRP1-coated hydroxyapatite (HAP) beads by 50 and 83%, respectively. However, the N-terminal fragment failed to show any effect. Analogous peptides corresponding to residues 75 to 89, 90 to 106, 107 to 120, 121 to 129, and 130 to 150 of PRP1 were synthesized. The fimbriae significantly bound **peptide** 130-150, **immobilized** on **96-well plates**, and the peptide also inhibited binding of

125I-labeled fimbriae to PRP1-coated HAP beads by almost 100%. Peptides 75-89, 90-106, and 121-129, immobilized on **plates**, showed considerable ability to bind fimbriae. For further analysis of active sites in residues 130 to 150, synthetic peptides corresponding to residues 130 to 137, 138 to 145, and 146 to 150 were prepared. Peptide 138-145 (GRPQGPPQ) inhibited fimbrial binding to PRP1-coated HAP beads by 97%. This amino acid sequence was shared in the alignment of residues 75 to 89, 90 to 106, and 107 to 120. Six synthetic peptides were prepared by serial deletions of individual residues from the N and C termini of peptide GRPQGPPQ. Peptide PQGPPQ was as inhibitory as peptide GRPQGPPQ. Further deletions of the dipeptide Pro-Gln from the N and C termini of peptide PQGPPQ resulted in significant loss of the inhibitory effect. These results strongly suggest that PQGPPQ is the minimal active segment for binding to P. gingivalis fimbriae and that the moiety of the Pro-Gln dipeptide plays a critical role in expressing binding ability.

L21 ANSWER 14 OF 24 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 1998:20135 SCISEARCH
THE GENUINE ARTICLE: YM293
TITLE: A high throughput enzyme-linked immunosorbent assay for inhibitors of the interaction between retinoblastoma protein and the Leu-X-Cys-X-Glu motif
AUTHOR: Ellsmore V A; Teoh A P; Ganesan A (Reprint)
CORPORATE SOURCE: NATL UNIV SINGAPORE, INST MOL & CELL BIOL, CTR NAT PROD RES, 15 LOWER KENT RIDGE RD, SINGAPORE 119076, SINGAPORE (Reprint); NATL UNIV SINGAPORE, INST MOL & CELL BIOL, CTR NAT PROD RES, SINGAPORE 119076, SINGAPORE; UNIV BATH, DEPT BIOCHEM, BATH BA2 7AY, AVON, ENGLAND
COUNTRY OF AUTHOR: SINGAPORE; ENGLAND
SOURCE: JOURNAL OF BIOMOLECULAR SCREENING, (WIN 1997) Vol. 2, No. 4, pp. 207-211.
Publisher: MARY ANN LIEBERT INC PUBL, 2 MADISON AVENUE, LARCHMONT, NY 10538.
ISSN: 1087-0571.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 20
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A **96-well** enzyme-linked immunosorbent assay was developed to discover compounds that inhibit the binding of the Leu-X-Cys-X-Glu (LXCXE) motif to the retinoblastoma tumor suppressor protein (pRB). The assay uses a LXCXE-containing multiple antigenic **peptide** (MAP) which is **immobilized** on a microtiter **plate**. A truncated form of pRB is added and the amount bound detected by a monoclonal antibody. This rapid assay was employed in high throughput screening of crude natural product extracts and discrete compounds.

L21 ANSWER 15 OF 24 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 96219975 MEDLINE
DOCUMENT NUMBER: 96219975 PubMed ID: 8639899
TITLE: Ligation of CD69 induces apoptosis and cell death in human eosinophils cultured with granulocyte-macrophage colony-stimulating factor.

AUTHOR: Walsh G M; Williamson M L; Symon F A; Willars G B; Wardlaw A J
CORPORATE SOURCE: Department of Respiratory Medicine, Leicester University School of Medicine, Glenfield Hospital, UK.
SOURCE: BLOOD, (1996 Apr 1) 87 (7) 2815-21.
Journal code: A8G; 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960726
Last Updated on STN: 19960726
Entered Medline: 19960718

AB Peripheral blood (PB) eosinophils rapidly undergo apoptosis and cell death in vitro unless cultured in the presence of cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) in which their survival is prolonged for up to 10 days. CD69 is a type II membrane antigen expressed by cytokine-activated, but not freshly isolated, PB human eosinophils. We have examined the effect of ligation of CD69 by specific monoclonal antibody (MoAb) on the viability of human eosinophils cultured with recombinant human (rh)GM-CSF. Eosinophils were purified by immunomagnetic selection and cultured with GM-CSF (10(-10) mol/L). Eighteen hours after the start of culture, a panel of CD69 MoAb or controls (anti-CR3 or isotype-matched control MoAb) were added. Viability was assessed by trypan blue exclusion and apoptosis by morphologic assessment, DNA laddering, and flow cytometric analysis of eosinophil red autofluorescence. Up to 50% of the eosinophils had undergone apoptosis 48 hours after addition of anti-CD69 MoAb compared with less than 10% apoptosis for CR3 or the isotype matched control. The majority of apoptotic eosinophils excluded trypan blue at 48 hours post CD69 ligation. More apoptotic eosinophils were observed at later time-points and this was associated with loss of viability. At 120 hours post-addition of the anti-CD69 MoAb MLR3, 24% +/- 10.6% eosinophils were viable compared with 84% +/- 3.4% for the CR3 control ($P < .001$). A F(ab)2 fragment of CD69 MoAb P8, also induced apoptosis in GM-CSF cultured eosinophils. A more rapid induction of eosinophil apoptosis was obtained with CD69 MoAb **immobilized** via their Fc portions on **protein**-A coated plastic **96 well plates**. Ligation of CD69 or CR3 resulted in the release of comparable quantities of eosinophil peroxidase at 48 hours post-ligation. These levels of EPO were consistent with the viability of these cells at 48 hours as assessed by exclusion of trypan blue. Finally, a neutralizing MoAb to TGF beta 1 had no effect on CD69-dependent apoptosis induction nor were there detectable quantities of TGF beta 1 in supernatants from GM-CSF--cultured eosinophils ligated with CD69 or control MoAb. These results suggest that eosinophils cultured with GM-CSF can be induced to undergo apoptosis as a result of cell signalling mediated by perturbation of CD69. This may represent an important physiologic mechanism for eosinophil removal in vivo.

L21 ANSWER 16 OF 24 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 95227832 MEDLINE
DOCUMENT NUMBER: 95227832 PubMed ID: 7712302
TITLE: The adhesion of Helicobacter pylori extract to four

mammalian cell lines.

AUTHOR: Ho B; Jiang B
CORPORATE SOURCE: Department of Microbiology, National University of Singapore.

SOURCE: EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY, (1995 Feb) 7 (2) 121-4.
Journal code: B9X; 9000874. ISSN: 0954-691X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950524
Last Updated on STN: 19950524
Entered Medline: 19950512

AB OBJECTIVE: To study the adherence of acid-glycine extract (AGE) from Helicobacter pylori to four mammalian cell lines: KATO III, CCL17, CCL156 and Neuro-2A. DESIGN: In vitro assays to assess H. pylori adherence were based on the principle of the affinity of the bacterial antigens to mammalian cells in culture. Protein extracts from mammalian cells were either coated onto **96-well** microtitre **plates** or electrophoretically resolved and blotted onto **immobilon**-P membranes. When the adhesive **proteins** derived from the AGE of H. pylori adhered to the mammalian cell proteins, these adhesive antigens were detected by rabbit anti-H. pylori AGE antibodies. METHODS: The adhesive proteins in H. pylori NCTC11637 AGE were detected by enzyme-linked immunosorbent assay (ELISA) and Western blot under non-denaturing conditions. RESULTS: Adhesive proteins constitute 5% H. pylori AGE proteins. The adhesive proteins adhered more readily to mammalian cell extracts than to bovine serum albumin. Modified Western blot analysis showed their affinity to a number of mammalian proteins ranging in molecular weight from 40 to 900 kDa, and in particular to the 140-230 kDa proteins. CONCLUSIONS: The two in vitro adherence assays used effectively demonstrated the adherence of H. pylori to different mammalian cell lines. The 5% adhesive proteins from H. pylori may play an essential role in infection and colonization by H. pylori. Furthermore, adhesive proteins have similar adherence properties to mammalian cell components and may play an important role in the pathogenesis of H. pylori infection.

L21 ANSWER 17 OF 24 JAPIO COPYRIGHT 2002 JPO
ACCESSION NUMBER: 1994-032800 JAPIO
TITLE: **PROTEIN-IMMOBILIZED** CARRIER AND SEPARATION OF CELL USING THE SAME
INVENTOR: OOHARA TAKAAKI; SUMIYA TORU
PATENT ASSIGNEE(S): KANEGAFUCHI CHEM IND CO LTD, JP (CO 000094)
PATENT INFORMATION:

PATENT NO	KIND	DATE	ERA	MAIN IPC
JP 06032800	A	19940208	Heisei	(5) C07K017-04

JP
APPLICATION INFORMATION
ST19N FORMAT: JP1992-187839 19920715
ORIGINAL: JP04187839 Heisei
SOURCE: PATENT ABSTRACTS OF JAPAN, Unexamined

Applications, Section: C, Sect. No. 1199, Vol. 18, No. 251, P. 41 (19940513)

AN 1994-032800 JAPIO

AB PURPOSE: To provide a lymphotoxin-immobilized carrier for therapies, etc., capable of selectively adsorbing lymphotoxin receptor-bearing cells thereto, thus capable of separating and eliminating leukocytes from the blood for transfusion or the blood of patients with rheumatism or autoimmune diseases.
CONSTITUTION: A lymphotoxin solution produced from Chinese hamster ovary cells by stimulating lymphocytes with a phorbol ester or mitogen, etc., or stimulating established lymphocyte-derived cells with e.g. mitogen, or by using a recombinant gene prepared with a cloned lymphotoxin CDNA, is incorporated with a 0.1M glycine buffer solution (pH 8.2) containing 0.15M NaCl, and the resultant solution is added to the wells of a carrier such as a plastic **96-well plate** at 100.mu.l per well and then allowed to stand overnight at 4.degree.C followed by addition of a 1% bovine serum albumin solution to effect blocking, thus obtaining the objective lymphotoxin-immobilized carrier capable of specifically binding lymphotoxin receptor-bearing cells thereto.

L21 ANSWER 18 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:448557 BIOSIS
DOCUMENT NUMBER: PREV199497461557
TITLE: Detection of Pseudomonas fluorescens and related psychrotrophic bacteria in refrigerated meat by a sandwich ELISA.
AUTHOR(S): Gonzalez, I.; Martin, R.; Garcia, T.; Morales, P.; Sanz, B.; Hernandez, P. E.
CORPORATE SOURCE: Dep. Nutr. Bromatol. III, Fac. Vet., Univ. Complutense, 28040 Madrid Spain
SOURCE: Journal of Food Protection, (1994) Vol. 57, No. 8, pp. 710-714.
ISSN: 0362-028X.
DOCUMENT TYPE: Article
LANGUAGE: English

AB A sandwich enzyme-linked immunosorbent assay (ELISA) was developed for detection of Pseudomonas fluorescens and related psychrotrophic bacteria in refrigerated meat. Polyclonal antibodies were raised in rabbits against protein F from the cell envelope of P. fluorescens AH-70. The ELISA involved capturing antigens from the microorganisms present on meat samples with the anti-**protein** F antibodies **immobilized** on **96-well plates**, and detecting bound antigens with the same anti-PF antibodies conjugated to biotin. Commercial ExtrAvidin-peroxidase conjugate was used to detect the biotinylated antibodies bound to their specific antigens. Subsequent enzymatic conversion of substrate gave distinct absorbance differences when assaying meat samples containing P. fluorescens strains of different origin as well as related psychrotrophic microorganisms. The detection threshold for the ELISA assay developed in this work is 10-5 CFU/cm-2.

L21 ANSWER 19 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:389817 BIOSIS
DOCUMENT NUMBER: PREV199497402817
TITLE: Detection of goats' milk in ewes' milk by an indirect ELISA.
AUTHOR(S): Garcia, T. (1); Martin, R.; Rodriguez, E.; Morales,

P.; Gonzalez, I.; Sanz, B.; Hernandez, P. E.
CORPORATE SOURCE: (1) Dep. Nutr. Bromatol. III, Fac. Vet., Univ. Complutense, 28040 Madrid Spain
SOURCE: Food and Agricultural Immunology, (1994) Vol. 6, No. 1, pp. 113-118.
ISSN: 0954-0105.
DOCUMENT TYPE: Article
LANGUAGE: English
AB An indirect ELISA has been developed successfully for the detection of defined amounts of goats' milk (1-100%) in ewes' milk. The assay uses polyclonal antibodies against goats' whey proteins (GWP) raised in rabbits. The anti-GWP antibodies were recovered from the crude antiserum by immunoadsorption and elution from a column containing immobilized GWP. The anti-GWP antibodies were biotinylated and rendered goats' milk-specific by mixing them with lyophilized cows' and ewes whey proteins. ExtrAvidin-peroxidase was used to detect the specific anti-GWP antibodies bound to goats' milk **proteins immobilized** on **96-well plates**.
Subsequent enzymic conversion of substrate resulted in discernible differences in optical density between mixtures of ewes' milk containing variable amounts of goats' milk.

L21 ANSWER 20 OF 24 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 92347390 MEDLINE
DOCUMENT NUMBER: 92347390 PubMed ID: 1379182
TITLE: A method for rapid screening of recombinant proteins for recognition by T lymphocytes.
AUTHOR: Hickling J K; Jones K R; Yuan B; Rothbard J B; Bulow R
CORPORATE SOURCE: ImmuLogic Pharmaceutical Corporation, Palo Alto, CA 94304.
SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, (1992 Aug) 22 (8) 1983-7.
Journal code: EN5; 1273201. ISSN: 0014-2980.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 19920911
Last Updated on STN: 19970203
Entered Medline: 19920901
AB A simple, cost-effective method is described that allows rapid screening of recombinant protein sequences for their ability to stimulate T cells. Individual microcultures of E. coli each expressing a gene product or peptide sequence fused to protein A are grown in **96-well plates**. Following lysis of the bacteria, the fusion **peptide** is readily captured with **immobilized** immunoglobulin in tissue culture wells. No further purification is required. T lymphocytes plus appropriate antigen-presenting cells are added directly to the wells and assayed for proliferation. The DNA in bacteria from wells stimulating T cell proliferation is then sequenced. The technique allows rapid mapping of T cell epitopes by facilitating screening of truncation mutants without extensive purification. Described here is a further application of the technique to study monosubstituted analogues of a known T cell epitope.

L21 ANSWER 21 OF 24 MEDLINE
ACCESSION NUMBER: 93018142 MEDLINE
DOCUMENT NUMBER: 93018142 PubMed ID: 1383356
TITLE: Development of serodiagnostic kit "HITAZYME Chlamydia Ab" for Chlamydia trachomatis infections using extracted antigen.
AUTHOR: Matsumoto A; Bessho H; Kishimoto T; Soejima R; Watanabe H; Kawagoe K
CORPORATE SOURCE: Department of Microbiology, Kawasaki Medical School, Kurashiki, Japan.
SOURCE: KANSENSHOGAKU ZASSHI. JOURNAL OF THE JAPANESE ASSOCIATION FOR INFECTIOUS DISEASES, (1992 May) 66 (5) 584-91.
Journal code: IJR; 0236671. ISSN: 0387-5911.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19960129
Entered Medline: 19921112

AB To develop EIA kit with low cross-reactivity for the quantitative detection of anti-chlamydial antibodies, we examined the preparation of trachomatis antigens and its specificity to mouse antisera and human sera. The chlamydial elementary body (EB) purified from C. trachomatis L2/434/Bu strain was treated by Sarkosyl, dithiothreitol and SDS by turns to obtain the soluble EB outer membrane (COMC). SDS-PAGE showed that the major components of the COMC were 96K, 60K and 39.5 KDa **peptides**. The reactivity of the COMC **immobilized** to **96 wells** microtiter **plate** to mouse anti-serum to C. trachomatis was higher than the other two mouse anti-sera to C. psittaci and pneumoniae. In human sera, the cut off values were calculated from an average optical density plus its two-fold standard deviation obtained by the testing of 100 samples of healthy human sera. We evaluated the specificity of the kit to 17 anti-C. pneumoniae, 9 C. trachomatis and 4 C. psittaci antibodies positive patients' sera judged by the MFA method respectively. The results showed that the concordance ratio of IgG and IgA were 88%, 100% in anti-C. pneumoniae, 89%, 78% in anti-C. trachomatis and 50%, 50% in anti-C. psittaci respectively. From the results obtained in this study, we concluded that the HITAZYME method which had a very low cross-reactivity to C. pneumoniae is clinically useful in the serodiagnosis of C. trachomatis infections, even if it has a little common antigenicity with C. psittaci antigen.

L21 ANSWER 22 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1992:433013 BIOSIS
DOCUMENT NUMBER: BA94:85138
TITLE: INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF COW'S MILK IN GOAT'S MILK.
AUTHOR(S): CASTRO C; MARTIN R; GARCIA T; RODRIGUEZ E; GONZALEZ I; SANZ B; HERNANDEZ P E
CORPORATE SOURCE: DEP. NUTR. BROMATOL. III, FAC. VET., UNIV. COMPLUTENSE, 28040 MADRID, SPAIN.
SOURCE: FOOD AGRIC IMMUNOL, (1992) 4 (1), 11-18.
CODEN: FAIMEZ.

FILE SEGMENT: BA; OLD
LANGUAGE: English
AB An indirect enzyme-linked immunosorbent assay (ELISA) has been developed for the specific detection of cow's milk (1-25%) in goat's milk. The test uses polyclonal antibodies raised in rabbits against bovine whey proteins (BWP). The anti-BWP antibodies were recovered from the crude antiserum by immunoadsorption and elution from a column containing immobilized BWP. The anti-BWP antibodies were biotinylated and rendered cow's milk specific by mixing them with lyophilized ovine and caprine whey proteins. Streptavidin-peroxidase was used to detect the biotinylated antiBWP antibodies bound to bovine milk **proteins immobilized** on **96 -well plates**. The colour developed by the subsequent enzymic conversion of the substrate gave clear absorbance differences when assaying mixtures of goat's milk containing variable amounts of cow's milk.

L21 ANSWER 23 OF 24 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1991-180585 [25] WPIDS
DOC. NO. NON-CPI: N1991-138201
DOC. NO. CPI: C1991-077914
TITLE: Measuring anti-adult T-cell leukaemia virus antibodiesitoring - using synthesised insolubilised antigen immobilised on insol. carrier.
DERWENT CLASS: B04 S03
PATENT ASSIGNEE(S): (NISE-N) NIPPON SEKIJUJISHA
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 03107764	A	19910508	(199125)*		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 03107764	A	JP 1989-245463	19890921

PRIORITY APPLN. INFO: JP 1989-245463 19890921
AN 1991-180585 [25] WPIDS
AB JP 03107764 A UPAB: 19930928
In a method for the measurement of the antibody (I) in which the component peptides of protein constituting adult type T cell leukemia virus (HTLV) is synthesised and at least one of the synthesised **peptides** is **immobilised** on an insoluble carrier to give an insolubilised antigen. The antigen is used for the measurement.

USE/ADVANTAGE- The method is simple and highly precise for the detection of anti-HTLV antibody, HTLV antibodies can be screened in a one step operation.

In an example, 27 peptides of various amino acid sequences are synthesised based on an assumption of the nucleic acid sequence of gag gene and env gene of HTLV-1. The synthesis is carried out by using t-butoxy-carbonylaminoacyl -4-(oxymethyl)-pam resin as the solid phase. The peptides are dissolved in 0.01M NaHCO3 buffer and applied on a **96-well** EIA **plate**, which